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Agricultural Experiment Station

Physiologic Specialization and Parasitism of Helminthosporium sativum

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PHYSIOLOGIC SPECIALIZATION AND PARASITISM OF HELMINTHOSPORIUM SATIVUM[1]

By J. J. Christensen

INTRODUCTION

The *"Helminthosporium* disease" of cereals, especially of wheat and barley, caused by *Helminthosporium sativum* P. K. and B. is prevalent and destructive every season in Minnesota. Moreover, it is widely distributed and causes serious losses. The writer has isolated the organism from material obtained from many states from Maine to Montana and Minnesota to Texas; also, from material obtained from several places in Mexico and Canada, and from one locality in Serbia. Other investigators (31 and 37) have reported the fungus from Canada, Argentina and Australia. Stakman (60), Christensen (18), Dosdall (24), and several other workers have called attention to the destructiveness of this disease, altho it is difficult to obtain accurate figures on losses.

Previous investigations have shown that the pathogene reduces crop yields in several ways. It lowers the percentage of seed germination, causes stunting and damping-off of seedlings, and premature death of ripening plants. It blights the seeds and floral parts and attacks the rachis and rachilla, thus preventing the caryopsis from developing normally. It causes severe spotting of leaves, stems, and glumes; and rots the roots, nodes, and basal portions of the stems.

Christensen and Stakman (20) pointed out that in 1924, in the hard red spring wheat region, *H. sativum* was the principal cause of premature death of wheat plants after heading. The disease was especially widespread and destructive in Minnesota. A disease survey of the state indicated that root- and basal stem-rots killed from 2 to 4 per cent of the plants of common wheat after heading, and from 5 to 15 per cent of durum wheat plants. In many fields of durum from 20 to 30 per cent of plants were killed, and in several others as many as 75 per cent had succumbed. A study of the parasitism of *H. sativum* is of practical importance and scientific interest, because the organism is a menace to wheat and barley culture and because it consists of many physiologic forms which mutate readily, especially on culture media (19). It is obvious that a knowledge of the number, cultural characteristics, parasitic capabilities, stability, and geographic distribution of physiologic forms is the essential basis for the development of permanent control

methods. It is especially important to obtain more information regarding the cause and frequency of mutations and variations, and of the behavior of the mutants in culture media and on host plants. It is important, also, to determine the effect of environmental factors on the development of the pathogene and on the susceptibility of host plants.

It is becoming axiomatic that several things must be known about a pathogene and its host plants in order really to understand the course of development of any plant disease.

The first things to understand thoroly are the pathogene and the factors affecting its development. Physiologic specialization has been discovered in so many different pathogenic fungi that one of the requisites in studying any pathogene is to determine the number and characteristics of physiologic forms. Furthermore, environmental factors are known to affect profoundly the behavior and structure of different physiologic forms (18, 43, and 57).

Even a routine study of a plant disease must therefore take into consideration a study of the physiologic specialization and the factors affecting the development of the pathogene. But the virulence and variability of the pathogene are only one phase of the problem. Host plants themselves may be greatly affected by the conditions under which they are growing. It is known that certain environmental factors may predispose plants to disease in a very real sense (22 and 27). Consequently, it also is important to know which factors render the plant more susceptible and to what degree the susceptibility can be changed by these environmental factors.

All these phases are important, and one should not be emphasized at the expense of the others. It must be realized that a disease is the result of the interaction between a pathogene and a host plant, both of which are living organisms with certain inherent and inherited tendencies which depend on the environment for their expression. The investigation was planned with these general principles in mind.

OBJECTS OF INVESTIGATION

The objects of the investigation were:

1. To ascertain the number and distribution of physiologic forms of *H. sativum*.

2. To ascertain the possible differences in virulence or pathogenicity of the different forms.

3. To determine the degree of stability of these forms under varying cultural conditions.

4. To investigate the effect of environmental factors on germination and vitality of spores.

5. To determine the varietal resistance of wheats to *H. sativum*.

6. To determine the effect of environmental conditions on the pathogenicity of *H. sativum* on wheat and barley.

7. To study the factors which predispose host plants to *H. sativum*.

8. To devise control methods other than the use of resistant varieties.

STUDIES ON GEOGRAPHIC DISTRIBUTION AND PREVALENCE

It has already been shown by Stakman (60), Christensen (18), and others that *H. sativum* is generally prevalent on small grains and grasses in the United States. It has been shown that the pathogene is abundant in many seed lots of wheat, barley, and rye (18 and 37). But it is logical to expect differences in the prevalence of the pathogene for a given region in different years and in different regions in the same year. For this reason the writer collected material from as many sources as possible, in order to obtain additional data on the geographic distribution and prevalence of the organism. This material consisted of infected seed lots, root systems, and culms of different varieties of grains. The method employed for isolation of the organism from various plant parts was similar to that already described by Christensen (18). Thousands of isolations of *Helminthosporium* were secured from various portions of small grains and numerous wild grasses.

There is a great variation in the percentage of seeds of wheat, rye, and barley infected with *H. sativum*. The amount of infection varies with locality, year, and variety of grain. At University Farm, St. Paul, Minn., durum wheat seed is usually more generally infected than the seed of common varieties. There also are striking variations in the percentage of seed infection by different organisms in different localities. Thus, in 1923, *Fusarium* predominated on seeds of Marquis wheat in southern Minnesota, while *Alternaria* and *Helminthosporium* were more prevalent in the northern part of the state. Hoffer (38), Henry (37), and others have recorded great variations in the percentage of wheat seeds infected with different organisms. Henry (37) isolated species of 20 genera of fungi from wheat seed, but he concluded that *Helminthosporium* was the most common cause of blackpoint of wheat.

During the winter of 1922-23, isolations were made from seed of 59 varieties and strains of barley which had been sprayed at heading time with a spore suspension of *H. sativum*. The percentage of seed infection was based on the number of colonies that developed as a

result of plating-out on potato dextrose agar 50 kernels chosen at random from each of the 59 varieties. The inoculated barley varieties were sown the following spring in seven-foot rows at the rate of 7 grams of seed per row. These seed lots were sown on heavy and light soils, and notes were taken on the relative resistance of the different varities to root-rot, basal stem-rot, and foliage infection. The barley varieties used are listed in Table III in Minnesota Technical Bulletin 21 (33, pp. 7-9). The seed was obtained from the section of plant breeding of the University. The results are summarized in Table I.

Data on the degree of root and basal stem infection were taken when the plants were almost mature. Notes on the degree of leaf infection were taken somewhat earlier, while those on seed blight were procured after the grains were threshed.

The symbols H (heavy), M (medium), L (light), T (trace), and o (absence of infection) are used to denote degree of infection. Plus and minus signs are used to indicate slight variations within each class. In order to make more exact comparison, the various degrees of infection were designated by numbers as follows:

o $=1$	L$-=5$	M$-=8$	H$-=11$
T$-=2$	L $=6$	M $=9$	H $=12$
T $=3$	L$+=7$	M$+=10$	H$+=13$
T$+=4$			

The numerical method is the same as that used by Hayes, Stakman, et al. (33), except that the smaller figures indicate resistance and the larger ones susceptibility.

Colonies of *Helminthosporium* developed from seed of all varieties, whether resistant or susceptible. The average percentage of *Helminthosporium*-infected seeds was 45.6. Calculations were made to determine if there was any correlation between the percentage of *H. sativum* on barley seed isolated in 1922 and the average root and foliage infection of the plants from the same seed lots grown on sandy soil at Coon Creek and heavy soil at University Farm, in 1923. From the data given in Table II, an apparently significant correlation coefficient of $+0.2905 \pm 0.0804$ was obtained. However, the reduction in variability, determined by the formula $100 \times (1 - \sqrt{1 - r_2}) = 4.3$, i.e., the effect of seed infection on the subsequent development of the disease, is more or less incidental.

TABLE I

SUMMARY OF ISOLATIONS OF *Helminthosporium sativum* FROM SEEDS OF 59 VARIETIES AND STRAINS OF BARLEY WITH VARYING DEGREES OF RESISTANCE

Species and varieties	Nursery stock or culture No.	Percentage of seeds infected, 1922			Degree of root and foliage infection with Helminthosporium, 1923*				
		Total	Helmintho-sporium	Other fungi	Roots U. Farm	Roots Coon Creek	Foliage U. Farm	Foliage Coon Creek	Average
Hordeum vulgare									
Manchuria	Minn. 184	50	12	38	6	6	7	3	11
do	I-16-32	66	16	50	8	9	6	5	14
do	I-16-21	72	36	36	6	8	5	4	12
do	I-16-29	84	66	18	6	6	6	4	11
do	I-16-44	72	16	56	6	6	6	4	11
do	I-16-66	100	74	26	6	7	6	6	13
do	I-15-1	100	22	78	8	6	5	5	12
do	I-15-2	100	28	72	6	9	6	7	14
do	C 81	94	22	72	7	6	7	5	13
do	C 96	56	4	52	7	7	6	3	12
do	C 163	100	26	74	7	6	7	5	13
do	C 168	94	28	66	6	7	7	6	13
Trebi	III-20	96	70	30	10	10	10	6	15
do	I-16-14	100	33	64	8	9	7	6	18
Coast	III-20	100	90	10	9	12	6	6	17
Winter Club	III-20	...	...	...	6	8	9	9	16
Beldi	III-20	100	74	26	9	10	8	6	16
Mariout	III-20	100	70	30	9	9	13	6	18
Serbian	III-20	98	54	44	10	8	9	5	16
do	I-16-15	100	68	32	10	7	10	6	17
C. I. 894	I-16-84	78	24	54	10	7	6	4	14
Scotch	I-16-45	72	48	24	6	7	7	4	13
Sandrel	I-16-3	72	48	24	7	9	9	6	15
Odessa	I-18-2	94	66	28	6	8	8	6	13
Luth	C 93	100	8	92	7	6	7	5	13
Sandrel	C 104	100	80	20	10	9	6	6	16
Peruvian	III-20	100	58	42	11	7	9	5	16
Arequipa	C 267	100	74	26	10	12	10	8	20
Minsturdi	II-16-47	100	56	44	6	7	6	6	13
S. Afr. x Manch.	II-16-77	96	48	48	7	9	6	6	14
Highland Chief	I-16-31	100	38	62	10	12	8	5	18
Smooth Awn	C 284	100	42	58	9	10	8	6	17
Bay Brewing	I-18-3	100	68	32	9	10	9	9	19
Gatami	III-20	92	80	12	12	11	5	7	18
Lion	III-20	100	64	36	11	12	10	6	20

TABLE I—Continued

Species and varieties	Nursery stock or culture No.	Percentage of seeds infected, 1922			Degree of root and foliage infection with Helminthosporium, 1923*				
		Total	Helmintho-sporium	Other fungi	Roots		Foliage		Average
					U. Farm	Coon Creek	U. Farm	Coon Creek	
Hordeum vulgare									
Lion	I-16-13	88	54	34	11	12	9	7	20
Lion	C 270	100	70	30	9	10	9	7	18
Horsfords	III-20	100	42	58	7	9	8	6	15
H. v. horsfordianum	....	100	64	36	9	5	9	5	14
H. v. aethiops	....	100	56	44	7	7	7	5	13
H. v. coeleste	....	96	48	48	8	7	9	6	15
Black Hull-less	....	70	30	40	9	10	6	6	16
Nepal	III-20	26	8	18	9	7	8	3	14
Hordeum intermedium									
H. i. nudihaxtoni	....	62	20	42	11	7	12	7	19
H. i. cornutum	....	70	20	40	12	13	12	8	23
Hordeum distichon									
Hanna C. I. 906	III-20	100	50	50	6	9	6	6	14
Hanna	III-20	100	26	74	8	7	5	6	13
Haunchen	III-20	100	68	32	9	10	7	5	16
Boltons	III-20	88	12	76	9	8	5	5	14
Svanhals	I-13-21	100	58	42	6	8	6	6	13
Svansota	II-16-37	100	72	28	6	6	7	6	13
Chevalier	Minn. 230	100	74	26	6	7	5	6	12
Chevalier	III-20	..	..	..	6	9	8	6	13
H. d. n. persicum	C. I. 1003	100	70	30	8	12	8	6	17
do	S. P. I. 38316	100	54	46	9	12	8	7	18
Poppenheim	....	66	36	30	6	9	7	6	15
H. d. nigrinudum	....	..	..	..	12	12	6	6	18
Hordeum deficiens									
H. def. deficiens	C. I. 6684	74	7	67	7	6	11	6	15
do	....	100	38	62	6	6	12	9	17
H. def. steudelii	....	100	38	62	6	6	12	7	16
H. def. tridax	....	96	22	74	7	6	12	7	16
H. def. nudideficiens	....	100	46	54	12	13	9	6	20
Average	....	90.2	45.6	44.6	8.1	8.4	7.7	5.8	15.0

* 1 = No infection
2 = Trace—
3 = Trace
4 = Trace+
5 = Light—
6 = Light
7 = Light+
8 = Moderate—
9 = Moderate
10 = Moderate+
11 = Heavy—
12 = Heavy
13 = Heavy+

TABLE II

CORRELATION BETWEEN PERCENTAGE OF SEEDS OF 59 VARIETIES OR STRAINS OF BARLEY INFECTED
WITH *Helminthosporium sativum* AND THEIR AVERAGE RESISTANCE FOR 1923 WHEN
GROWN AT COON CREEK AND UNIVERSITY FARM

	Percentage of seed infected in 1922									
Average reaction at University Farm and Coon Creek, 1923	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	
11		2		1			1			4
12	1		1					1		3
13	1		4		1	3 .	1	2		12
14	1	2	2	.	2		1			8
15	1			2	3					6
16			2	1		2	1	2		8
17				1	1		2		1	5
18				1		1	3	1		6
19		1					1			2
20					1	1	1	1		4
21										0
22										0
23			1							1
	4	5	•10	6	8	7	11	7	1	59

$r = +.2905 \pm .0804$

In order to ascertain the variability in the amount of *Helmintho-sporium* infection due to chance, selection of infected seed, or environmental conditions in the same plots, isolations were made from nine replicated five-foot rows of the *Helminthosporium*-resistant Manchuria barley (Minn. No. 184). Fifty seeds from each row were sown on agar, as above. The variability which occurred is summarized in Table III. Thus, within a single variety of barley described as highly resistant (33), the *Helminthosporium*-infected seeds varied from 12 to 66 per cent, with an average of 42.8 per cent.

TABLE III

VARIABILITY IN PERCENTAGE OF SEEDS OF MANCHURIA BARLEY (MINN. No. 184) INFECTED
WITH *H. sativum*, GROWN IN REPLICATED FIVE-FOOT ROWS IN 1922,
AND THE MEAN *Helminthosporium* REACTION OF THESE
ROWS AT UNIVERSITY FARM AND COON CREEK IN 1923

No. of test	Percentage of seeds infected with Helminthosporium	Helminthosporium figure* average for University Farm and Coon Creek
1	12	11
2	42	13
3	44	12
4	54	14
5	66	12
6	66	12
7	48	12
8	14	13
9	40	11
Average	42.8	12.2

* Sum of averages of foliage and root infection. For explanation of figures, see footnote to Table I.

These results are in accord with the conclusions of Hayes, Stakman, et al. (33), that resistance is relative, not absolute; that the roots, foliage, and spikes of all varieties of barley may be infected, but in varying degrees. The degree of spike blighting is not correlated with the presence of the organism on the seed, but rather with the inherent susceptibility of the host.

Helminthosporiuan and Alternaria spp. constituted nearly all the fungi isolated from the barley seed. Relatively few colonies of *Fusarium* developed. It must be remembered that 1922 was not a bad scab year, at least very little scab developed in the vicinity of St. Paul. This perhaps accounts for the small percentage of *Fusarium*-infected seeds. A few other species of fungi belonging to different genera were observed. Several apparently new and distinct forms of *H. sativum* were secured.

Brownish lesions always developed on seedlings from kernels infected with *H. sativum* grown on agar, and these seedlings were eventually killed by the pathogene. *Alternaria spp.* were never observed to cause any decided browning while the plates were under observation.

Isolations from barley seeds procured from widely different sources indicated that species of fungi belonging to several genera can cause blighting. Inoculation experiments proved that at least 5 or 6 apparent species of *Helminthosporium* and one species of *Brachysporium,* besides numerous physiologic forms of *H. sativum,* can cause blighting of barley spikelets and grains. All species and forms concerned produce very similar discoloration on the spikelets and kernels. The relative virulence of the various forms of *H. sativum*—spike-blighting and root- and foot-rotting organisms—will be discussed later.

During the summer of 1924 there was considerable killing of wheat plants after heading (20). On account of the destructiveness of this disease, numerous isolations were made to determine the fungi responsible. The results are recorded in Table IV. *H. sativum* was by far the most prevalent organism on wheat. *Fusarium spp.* were more common on durum varieties than on common and emmer varieties or hybrids. Durums appear to be more susceptible to *Fusarium spp.* This is probably due to the fact that many of the isolations from the durums were made after the plants had been dead for some time and secondary organisms had had an opportunity to gain entrance. In durum wheat which had been grown on peat soil inoculated with *H. sativum,* from 95 to 100 per cent of the plants were killed after heading. Of the colonies obtained from these plants, 87.5 per cent were *H. sativum,* 3.1 per cent *Fusarium,* and 9.4 per cent other fungi. As the disease symptoms in these plants were identical with those so general in the

field, it seems probable that *H. sativum* was principally responsible for the serious blight epidemic on wheat in Minnesota during the summer of 1924. However, it should be remembered that Bolley (8), Stakman (61), Henry (37), and others have demonstrated that several species of fungi belonging to different genera can cause root-rot of cereals.

TABLE IV

Summary of Results Obtained by Isolating Fungi from Basal Portions of the Culms of Cereals After Heading in 1924, in Minnesota

Cereal	Number of isolations	Percentage of fungi isolated		
		Helminthosporium*	Fusarium	Other fungi
Wheat				
Common wheats	468	77.8	13.1	9.1
Durums	390	61.0	26.4	12.6
Emmer	96	67.7	20.8	11.4
Durum x Common hybrids...	59	71.2	12.0	16.8
Total	1013	69.4	18.1	12.5
Oats				
Avena sativa	54	3.7	83.4	12.9

* Mostly *H. sativum.*

Altho *H. sativum* is so universally present on wheat and barley, it does not necessarily always cause appreciable damage. From Table I it will be seen that *H. sativum* was isolated from resistant as well as from susceptible varieties.

Isolations from oats indicate that *Fusarium spp.* probably were the primary cause of the blighting of oats after heading. Fields of oats were observed in Minnesota in which as many as 20 per cent of the plants were killed. These observations agree with the results of isolations made from oats seedlings during the last three years.

PHYSIOLOGIC SPECIALIZATION
NUMBER OF PHYSIOLOGIC FORMS

Helminthosporium sativum is a group species consisting of many elementary species or physiologic forms. In 1922, Stevens (62) and Christensen (18) showed that these forms differ in appearance and action on culture media and that differences in the size of spores of some forms can be detected by biometrical methods. Drechsler (26), Dosdall (24), and others also consider *H. sativum* a group species.

A systematic survey has never been made to determine the existing number of physiologic forms, and information regarding their pathogenicity, especially, is lacking. For these reasons the writer attempted to determine the number of forms and their characteristics.

It was found that they can be differentiated by the following criteria: cultural characteristics; rate of growth; thermal relations; amount of sporulation; zonation; the readiness with which they mutate; and, lastly, their pathogenicity.

All physiologic forms used in the following studies were derived from single spores. The method of procedure has been described elsewhere (19).

Stock cultures were grown for the most part on potato dextrose agar. These were kept either in the icebox at 7° to 10° C., or at room temperature, 18° to 23° C.

In Table V are given the form number, the host, the part of the plant from which isolated, the locality, the year, and the source of the culture for each of the 37 forms used.

More than 50 apparent forms were studied. Of these, 37 were studied in detail.

TABLE V

Sources of 37 Physiologic Forms of *Helminthosporium sativum*

Form	Original host	Plant part from which isolated	Locality	Year collected	Previous history*
1	Rye	Kernel	University Farm, St. Paul, Minn.	1919	Material collected by writer
2	Barley	Leaf	Duluth, Minn.	1920	Culture furnished by Louise Dosdall
3	Barley	Leaf	University Farm	1920	Material collected by writer
4	Wheat	Base of stem	Illinois		Culture furnished by F. L. Stevens, 1921
5	Wheat	Node	Saskatoon, Sask., Can.	1921	Culture furnished by A. W. Henry
6	Dactylis glomerata	Leaf	University Farm	1921	Material collected by writer
7	Agropyron repens	Leaf	Decorah, Iowa	1921	Material sent in
8	Corn	Seedling leaf	University Farm	1921	Material collected by writer
9	Wheat		Sydney, Australia		Culture furnished by C. O. Hamblin, 1921
10	Wheat	Base of stem	Edmonton, Alberta, Can.	1924	Material sent in by G. B. Sanford
11	Barley	Kernel	University Farm	1922	Material collected by writer
12	Barley	Leaf	Texas	1923	Material furnished by J. H. Martin
13	Wheat	Base of stem	Thief River Falls, Minn.	1921	Material collected by writer
14	Agropyron smithii	Leaf	University Farm	1921	Material collected by writer
15	Barley	Kernel	University Farm	1922	Material collected by writer
16	Wheat	Kernel	Argentine	1921	Culture furnished by A. W. Henry
17	Elymus striatus	Leaf	Minneapolis, Minn.	1921	Material collected by writer
18	Wheat		England		Culture furnished by F. T. Brooks
19	Wheat	Base of stem	Watertown, Minn.	1924	Material collected by writer

* Single spore isolations were made from all the cultures.

TABLE V—*Continued*

Form	Original host	which isolated Plant part from	Locality	collected Year	Previous history*
20	*Hordeum murinum*	Leaf	Serbia	1922	Material collected by E. C. Stakman
21	Wheat	Kernel	Ashby, Minn.	1923	Material sent in to Seed Laboratory
22	*Echinochloa crusgalli*	Leaf	University Farm	1921	Material collected by writer
23	Barley		New York	1923	Culture furnished by R. S. Kirby
24	Barley	Leaf	Duluth, Minn.	1921	Material collected by writer
25	*Setaria gluca*	Leaf	Waconia, Minn.	1921	Material collected by writer
26	*Echinochloa crusgalli*	Leaf	Iowa	1921	Material sent in
27	Barley	Kernel	University Farm	1922	Material collected by writer
28	Oats	Base of stem	Glencoe, Minn.	1924	Culture furnished by E. B. Lambert
29	Oats	Leaf	Maine	1924	Material sent in by R. Bonde
30	Wheat	Foot	Cape Town, Africa		Culture furnished by H. J. Hynes (originally from R. Davis) 1924
31	*Bromus inermis*	Leaf	Buffalo Lake, Minn.	1921	Material furnished by E. C. Stakman
32	Durum wheat	Stem	University Farm	1919	Culture furnished by Louise J. Stakman
33	Wheat	Base of stem	Bemidji, Minn.	1924	Material collected by writer
34	Barley	Kernel	University Farm	1922	Material collected by writer
35	Wheat	Base of stem	Oklahoma	1923	Material furnished by E. H. Ostrom
36	Oats	Base of stem	Crookston, Minn.	1924	Specimen sent in by J. G. Leach
37	Barley	Kernel	University Farm	1922	Material collected by writer

* Single spore isolations were made from all the cultures.

CULTURAL CHARACTERS OF DIFFERENT FORMS

In order to compare their cultural characters, the 37 forms were grown on two different media: a 1 per cent potato dextrose agar; and a similar medium in which oatmeal, rice, and cornmeal were substituted for the potato as a nutrient base. Some forms were grown on other media also. Triplicate plates of each medium were inoculated with each form. Small, and as nearly as possible equal, portions of medium containing mycelium, and usually also spores, were used as inoculum in each case. The plates were of uniform size and contained the same amount of medium, usually 18 cc. The agar was made up in one lot, tubed, sterilized, and poured at the same time. After inoculation, the plates were placed on the same table in the laboratory and kept at room temperature. Thus all were subjected to the same general environmental conditions. The triplicate colonies of the same

forms on the same medium were always alike, unless accidentally contaminated by other organisms. It was noticed that a small colony of bacteria sometimes profoundly affected the growth of certain forms. In the immediate vicinity of colonies of foreign organisms, sporulation was often stimulated, and the morphology of the mycelium was occasionally changed considerably.

The 37 forms listed in Table V can be differentiated on cultural media macroscopically by the following characters: rate of radial growth; relative amounts of submerged and aerial mycelium; nature of mycelial growth, whether woolly or cottony, etc.; zonation, whether lacking, prominent, moderate, or faint, and the frequency and distance apart of the zones; conidial production, whether absent, scarce, moderate, or abundant; conidial clusters; and color of mycelium from white to black with intervening gradations and tints of other colors. The length, width, shape, and septation of the conidia, also, are different in some of the forms.

Dosdall (24), Christensen (18), and Stevens (62) have shown that cultures of *H. sativum* grown on different media and on the same media but under different environmental conditions, were changed greatly in habit of growth, structure and appearance of colonies, and morphology of the spores. However, such changes are only temporary and are not inherited. This phenomenon has been observed by numerous workers in various saprophytic and parasitic organisms.

It is evident from Plates III and IV that cultural characters of different forms of *H. sativum* differ greatly from each other on the same medium. The cultural characters of the same forms also are different on different media. The colonies of many forms were so strikingly different in general appearance that one was prone to separate them into different species. However, their similarity on another medium forbade such a reclassification.

RATE OF GROWTH

The amount of growth of the forms on the two media was measured on the 3d, 6th, and 11th days after inoculation. The results are presented in Table VI. It has been shown previously (18) that there are marked variations in rate of growth of physiologic forms of *Helminthosporium* on the same and on different media. On potato dextrose agar, during a period of 11 days, Forms 15, 13, 19, and 23 increased in average diameters 8.7, 5.2, 2.2, and 1.6 millimeters respectively, while on oatmeal-rice-cornmeal agar the rate of increase for the same forms was 3.1, 5.2, 4.9, and 2.0 mm. respectively. It is apparent

from Table VI that one form may grow extremely rapidly on one medium and very slowly on another. Another form may behave in exactly the opposite manner on the same media (Plates III and IV).

TABLE VI

RATE OF GROWTH OF 37 PHYSIOLOGIC FORMS OF *Helminthosporium sativum* ON TWO DIFFERENT NUTRIENT MEDIA

Form No.	Diameter of colonies, mm.*									
	Oatmeal-rice-cornmeal agar					Potato dextrose agar				
	Age, days									
	3	6	8	11	Av.	3	6	8	11	Av.
1	16	31	38	47	4.3	15	35	45	58	5.3
2	17	33	35	45	4.1	17	41	55	71	6.4
3	16	31	37	45	4.1	11	22	37	46	4.2
4	24	41	47	50	4.5	16	22	23	31	2.8
5	21	54	59	37	3.3	12	30	38	48	4.4
6	19	31	36	44	4.0	19	39	56	66	6.0
7	21	62	88	96+	8.7+	8	25	35	52	4.7
8	25	45	54	63	5.7	15	36	50	68	6.2
9	17	26	30	38	3.4	12	20	27	33	3.0
10	15	26	35	40	3.6	17	46	56	74	6.7
11	21	52	69	95	8.6	12	24	33	45	4.1
12	28	36	45	57	5.2	13	30	37	48	4.3
13	17	39	48	57	5.2	14	33	40	58	5.2
14	25	58	67	82	7.4	19	33	43	58	5.2
15	21	52	70	96	8.7	7	14	20	34	3.1
16	16	31	39	46	4.2	13	35	48	65	5.9
17	20	37	43	50	4.5	18	34	43	55	5.0
18	12	26	34	57	5.2	13	32	41	56	5.1
19	10	16	20	24	2.2	15	30	40	54	4.9
20	11	15	19	24	2.2	12	27	34	45	4.1
21	24	67	86	96	8.7	10	30	45	58	5.2
22	18	32	38	46	4.2	9	27	38	51	4.6
23	12	14	15	17	1.6	6	14	18	22	2.0
24	16	26	33	40	3.6	13	32	43	59	5.4
25	15	27	36	46	4.2	12	31	44	64	5.9
26	22	59	83	95	8.6	17	44	58	73	6.7
27	18	28	39	54	4.9	14	33	44	55	5.0
28	17	26	32	42	3.6	17	33	41	53	4.9
29	16	29	35	45	4.1	16	34	45	58	5.3
30	19	24	27	31	2.9	15	31	41	54	4.9
31	14	24	30	37	3.4	16	33	43	58	5.3
32	19	37	43	50	4.6	14	33	48	69	6.3
33	16	25	29	33	3.0	10	17	19	26	2.4
34	17	27	33	40	3.7	12	30	45	62	5.6
35	17	36	48	63	5.8	17	36	46	61	5.5
36	17	32	33	41	3.8	15	21	27	36	3.3
37	20	31	36	48	4.4	13	34	43	60	5.5
Av.	18.1	34.8	42.9	51.8	4.7	13.6	30.3	40.2	52.9	4.8

* Measurements based on average of three colonies.

To determine the degree of correlation in rate of growth of the 11-day-old colonies of the 37 forms on the above-mentioned media, the correlation coefficient was computed. The results are given in Table VII.

TABLE VII

CORRELATION BETWEEN RATE OF GROWTH OF 37 FORMS OF *Helminthosporium sativum*, 11 DAYS OLD, ON POTATO DEXTROSE AGAR AND ON OATMEAL-RICE-CORNMEAL AGAR

		Growth on potato dextrose agar						
		21-30	31-40	41-50	51-60	61-70	71-80	
Growth on oatmeal-rice-cornmeal agar (in millimeters)	11-20	1						1
	21-30			1	1			2
	31-40	1	1	1	3	1	1	8
	41-50		1	1	6	4	1	13
	51-60		1	1	3			5
	61-70					2		2
	71-80							0
	81-90				1			1
	91-100		1	1	2		1	5
		2	4	5	16	7	3	37

$r + .1366 \pm .1089$

The calculated coefficient $+0.1366 \pm 0.1089$ is only slightly larger than its probable error. This indicates that the rate of growth of different forms is influenced differently by the kind and amount of nutrient.

The average rate of growth on both media, as well as pathogenicity tests, indicates that there is no correlation between the degree of virulence and the rate of growth on culture media. Thus, Form 23 made an average growth of 1.8 mm. per day for 11 days, altho it possesses a higher degree of virulence than Form 2, a relatively weak strain, which made a growth of 5.3 mm. per day. On the other hand, Form 11 grows more rapidly than Form 2, and is also more virulent.

The difference in rate of growth between slowly and rapidly growing forms on both media may be due partly to temperature relations. Dosdall (24) found that the minimum temperature for growth of *H. sativum* was near 0° to 2° C., the maximum between 35 and 39° C., and the optimum between 24° and 28° C. Preliminary tests with several forms indicate that the temperature relationship is not the same for all forms. For instance, Form 1 would not grow above 32° to 33 C., and grew very poorly at 29° to 30 C. Johnson (39) has recently shown that forms of *H. gramineum* Rab. are strikingly different in their cardinal temperature relationships.

ZONATION

The differences in zonation of different forms on potato dextrose agar and on oatmeal-rice-cornmeal agar are summarized in Table VIII. It is evident that zonation was pronounced in some forms, but lacking in others (Plates III and IV). In some forms zonation was due to variation in the quantity of conidia and to aerial mycelium or to both. Variation in color of mycelium often influenced the conspicuousness of the zones. The expression of color is influenced to a marked extent by temperatures and nutrients.

TABLE VIII

VARIABILITY IN NUMBER OF ZONES OF 37 FORMS OF *H. sativum* IN 11-DAY-OLD CULTURES GROWN AT ROOM TEMPERATURES

Form No.	Oatmeal-rice-cornmeal agar		Potato dextrose agar	
	No. of zones	Character of zones	No. of zones	Character of zones
1	8	Pronounced	7	Pronounced
2	8	do	9	do
3	8	do	5	Faint
4	5	do	7	Faint to pronounced
5	6	do	8	Faint
6	8	do	8	do
7	0	Absent	3	do
8	8	Pronounced	7	do
9	7	do	7	Faint to pronounced
10	8	do	7	Pronounced
11	6	Faint	6	Faint
12	8	Pronounced	6	Faint to pronounced
13	4	Faint	7	Faint
14	8	do	8	Faint to pronounced
15	7	do	0	Absent
16	8	Moderate	7	Faint
17	7	Pronounced	8	do
18	8	Mod. to pronounced	8	do
19	4	do	5	do
20	8	Pronounced	8	do
21	?	Moderate	4	do
22	8	Pronounced	6	Faint to pronounced
23	3	Faint to pronounced	0	Absent
24	8	Pronounced	6	Faint to pronounced
25	4	do	4	do
26	4	Faint	4	Faint
27	8	Pronounced	7	Faint to pronounced
28	8	do	7	do
29	8	do	7	do
30	4	do	8	Faint
31	8	do	8	Pronounced
32	7	Moderate	7	Faint to pronounced
33	8	Pronounced	4	Faint
34	7	do	8	Faint to pronounced
35	7	Moderate	5	do
36	8	Pronounced	6	Faint
37	8	do	7	Faint to pronounced

Zonation has been attributed to various causes (5, 17, 36, and 63) : short exposures to light, alternation of light and darkness, alternation of temperature, resting periods, staling products, mycelial crowding, alkaline media, and variation in amount of food.

Bisby (5) has reviewed previous studies and observations relating to zone formation in fungi and points out the lack of agreement among various workers. Bisby proved that zonation in cultures of *Fusarium discolor sulphureum* (Schl.) A. and W. could be caused by alternating light and temperature. He concluded that exposure of a culture of this organism to bright daylight for one-fourth to one-half second is sufficient to produce a zone. Exposure for 6 minutes to a twenty-five candle power carbon lamp at a distance of one meter was required to induce a noticeable ring, while exposure for about two minutes to a tungsten filament lamp of about the same candle power caused zone formation. Bisby states that "The light acts upon the outermost tips of the hyphae, and the phenomenon is, at least in part, another case of the effect of light upon growth." He induced ring formation by alternating temperature in constant darkness.

Chaudhuri (17), 1923, worked with *Verticillium albo-atrum* McA. and concluded that stale medium, one containing an old culture, and alkaline media, increased the sharpness of zones. Under certain conditions zonation could be prevented by raising the hydrogen-ion concentration. He also found that the organism produced zones in darkness only at $25°$ C. but, on exposing the cultures to constant light, zonation occurred at lower temperatures ($21°$ to $23°$ C.).

Data on the zonation of *H. sativum* in relation to environmental conditions are given in Tables IX and X. For the experiments summarized in Table IX, large test tubes, 25 x 2.5 cm. were used. Fifteen cc. of potato dextrose agar was poured into each tube and slanted so as to give a 15-cm. lateral surface, the center of which was inoculated. The tubes were then sealed by dipping the plugged end in melted paraffin in order to prevent drying out of the cultures and thus prolong the growing period. Duplicate cultures were made in every case. In making zonation tests the plate cultures were inoculated in the usual manner. Usually three or more plates were used for each set of conditions. In many cases the experiments were repeated several times. While the cultures were being interchanged they were necessarily exposed to daylight or lamplight. The controls were always exposed to the same light for the same length of time.

TABLE IX

RELATION OF TEMPERATURE AND LIGHT TO ZONATION OF *H. sativum* WHEN CULTURED IN LARGE TEST TUBES SEALED WITH PARAFFIN

Temperature, ° C.	Light relation	Zones
5-7 constant	Dark	o
10-12 do	do	o
16-18 do	Dark and diffused light (alternating)	o
21-22 do	Dark	o
24-25 do	do	o
29-30 do	do	o
32-33 do	do	o
20-22 do	Light (lamp)	o
5, 12, and 29 alternating*	Dark	+
12 and 29 alternating	do	+
18, 12, and 29 alternating	Dark and diffused light at 18, 12, and 29	+
32, 12, and 29 alternating	Dark	+
29 and 12 alternating	do	+
20 and 30 alternating every 12 hours	Diurnal change	+

* First figure represents temperature at which culture was first kept.

TABLE X

EFFECT ON ZONATION OF *H. sativum*, FORM 1, ON DIFFERENT MEDIA, AND VARIOUS AMOUNTS OF THE SAME MEDIUM, KEPT UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Medium		Tempera-	How		
Kind	Amount, cc.	ture, ° C.	kept	Light relation	Zones
Potato dextrose agar	30	5-7	Constant	Dark	o
do	20	5-7	do	do	o
do	20	10-12	do	do	o
do	30	24-25	do	do	o
do	20	24-25	do	do	o
do	20	24-25	do	do	Few zones at edge of old colonies
do	30	24 and 29	Alternating	do	+
do	20	24 and 29	do	do	+
do	20	20 and 30	do	do	+
do	20	20 and 30	do	Diurnal change	+
Oatmeal-rice-cornmeal agar	20	24-25	Constant	Dark	o
do	20	20 and 30	Alternating	do	+
do	20	24 and 12	do	do	+
do	20	20	Constant	do	Few zones at edge of old colonies
do	20	12 and 24	Alternating	Light	+
do	10	20-22	Constant	Dark	+
do	10	20-22	do	Diurnal change	+
do	15	20-22	do	Dark	+
do	15	20-22	do	Diurnal change	+
30	30	20-22	do	do	o
do	20	20-22	do	do	+ (weak)
Carrot agar	20	24-25	do	Dark	o, but one plate with zones at edge of old colony
do	20	24 and 29	Alternating	do	+
do	20	20 and 30	do	do	+

The exposure of cultures of *H. sativum*, Form 1, to daylight or to lamplight for from 5 to 15 minutes did not cause zonation, but alternating temperatures in constant light or darkness resulted in ring formation. A variation in temperature of 5° C. (25° to 30° C.) for 24 to 48 hours was sufficient to induce zonation. Alternation of temperature

from 20° to 30° C. at intervals of 10 to 12 hours resulted in zone formation. Under certain conditions alternation of diffused light and darkness at constant temperature also induced zonation. The exposure of cultures to variations in temperature from 20 to 30° C. in darkness, and to the same temperatures but to diurnal changes of light and darkness, resulted in the same number of zones. Those under the last named condition were much more distinct.

Increased depth of medium influenced the size and compactness of zones. Zones were produced in petri dishes containing from 10 to 15 cc. of oatmeal-rice-cornmeal agar, at constant temperature in darkness or in continuous light. But no zones developed in dishes containing 30 cc. of agar, unless the medium prevented rapid growth. In these cases zoning took place only after the culture had become one or two weeks old. However, no zonation occurred in test tubes, or in petri dishes when kept for several weeks at constant temperatures of 29°, 10°, and 6° C.

Thus it is apparent that the type of zone and the rate of formation vary strikingly on the same and on different media in different physiologic forms of *H. sativum*. Form 1 sometimes produced zones at constant temperature and in the absence of light in cultures, perhaps because of the production of staling substances by the fungus.

A change in temperature from one favorable to growth or sporulation of the organism to an unfavorable one caused the formation of conspicuous zones.

It is obvious, therefore, that the 37 physiologic forms of *H. sativum* behave differently physiologically, but the most important question is, Are they different pathogenically?

PATHOGENICITY OF PHYSIOLOGIC FORMS

Christensen (18), in 1921, noted variations in the degree of pathogenicity of certain physiologic forms of *H. sativum*. Dosdall (24) found a difference in degree of severity with which two strains of *H. sativum* attacked Lion barley and Marquis wheat. Henry (37) observed variation in virulence of strains of *H. sativum* obtained from different sources and was able to distinguish 4 strains of "small-spored Helminthosporia" by their differences in pathogenicity on wheat.

SOIL INOCULATIONS

The writer made tests of the comparative virulence of physiologic forms by inoculating soil in four 4-inch pots with pure cultures. Twenty-five seeds were planted in each pot, so that 100 seeds of each cereal variety were used in each test. The wheat seeds were treated by the Jensen modified hot-water treatment. The barley was soaked for 2 hours in a 0.25 per cent solution of Chlorophol, an organic mer-

cury compound. The soil used was a mixture of three parts garden loam and two parts sand. All the soil for a given experiment was mixed in one lot and then steamed for 3 hours. The inoculum was grown in Erlenmeyer flasks on autoclaved seed of wheat and oats in a proportion of three to one by volume. An equal amount of inoculum was added to each pot of a series. Even the forms that grew slowly on ordinary culture media grew well on this medium. The same quantity of uninoculated wheat and oats mixture was added to the controls. The pots were all placed on a center bench in the greenhouse in order that all might receive the same amount of heat and light.

Data on the comparative pathogenicity of 26 forms on Marquis wheat (C. I. 3641), and Mindum (Minn. No. 470), a durum variety, are given in Table XI. A record was made of the number of plants which emerged and of the number which developed beyond the two-leaf stage. Both varieties were more or less susceptible to all 26 forms of the pathogene, but there were distinct and consistent differences in the virulence of different forms on both varieties of wheat. Forms 5, 8, 11, 13, and 15 were the most virulent, while Forms 3, 19, 21, and 26 were relatively weak. There also are intermediates between the two extremes.

TABLE XI

RESULTS OF INOCULATING SOIL GROWING MARQUIS AND MINDUM WHEAT IN THE GREENHOUSE WITH 26 PHYSIOLOGIC FORMS OF *H. sativum*

Form No.	Percentage of seeds producing seedlings										Total No. of plants which survived	
	Marquis					Mindum						
	Pot No.					Pot No.						
	1	2	3	4	Av.	1	2	3	4	Av.	Marquis	Mindum
1	20	28	28	24	25.0	16	20	24	36	24.0	4	10
2	36	16	28	36	29.0	48	32	24	32	34.0	17	21
3	36	80	56	60	58.0	32	63	48	40	46.0	47	32
4	20	8	8	4	10.0	12	20	16	44	23.0	1	7
5	0	0	0	1	0.2	0	0	1	0	0.2	0	0
6	28	8	4	8	8.0	28	16	8	28	20.0	47	32
7	24	32	12	32	25.0	12	20	28	28	22.0	22	21
8	8	0	4	0	3.0	0	0	8	0	2.0	2	2
9	20	20	20	16	19.0	24	32	28	8	23.0	13	8
10	24	28	24	4	20.0	28	24	28	32	28.0	3	14
11	0	4	0	0	1.0	4	0	12	4	5.0	1	2
12	16	36	36	8	24.0	20	16	28	32	24.0	14	22
14	8	16	12	12	12.0	4	16	20	8	12.0	11	10
15	4	0	12	0	4.0	4	4	4	8	5.0	3	3
16	16	8	12	6	10.5	20	20	36	24	25.0	6	13
17	24	12	16	24	19.0	24	36	24	28	28.0	11	19
18	20	28	24	24	24.0	20	32	8	12	18.0	6	9
19	40	60	36	36	43.0	36	40	24	48	37.0	26	25
20	36	20	20	32	27.0	16	12	36	20	21.0	17	9
21	36	40	60	56	48.0	76	52	56	72	64.0	45	59
22	32	20	12	12	19.0	28	52	35	20	32.0	9	20
23	4	28	16	12	15.0	44	8	8	24	21.0	9	7
24	32	24	32	36	31.0	8	24	28	32	23.0	30	19
25	12	8	24	4	12.0	44	12	28	36	30.0	8	14
26	76	64	80	76	74.0	76	48	56	64	61.0	72	59
Control	88	84	90	92	86.0	88	84	80	80	83.0	86	83

There are also differences in the virulence of the different forms on varieties of barley. Trebi (C. I. 936) and Chevalier (C. I. 278) were inoculated with all the forms. The results are given in Table XII. Notes were taken on the percentage of plants which emerged and on the percentage of seedlings with deformed and infected primary leaves in each pot. The averages of these readings are given in Table XII.

TABLE XII

RESULTS OF INOCULATING SOIL GROWING TREBI AND CHEVALIER BARLEY IN THE GREENHOUSE WITH 26 PHYSIOLOGIC FORMS OF *H. sativum*

Form No.	Percentage of germination*		Percentage of seedlings with deformed primary leaves†		Degree of stunting‡	
	Trebi	Chevalier	Trebi	Chevalier	Trebi	Chevalier
1	90	89	37	37	L+	L+
2	89	89	31	37	M—	L+
3	88	93	17	16	T+	T+
4	88	88	7	14	L—	L
5	63	77	82	69	H	H
6	83	96	25	20	L	L
7	84	90	5	7	L—	L
8	76	85	59	33	M+	M
9	79	73	65	60	M—	M—
10	81	94	40	26	L+	L+
11	74	77	91	92	H—	M+
12	96	92	31	24	M—	L—
13	63	74	79	54	M—	L+
14	76	84	48	37	L+	L+
15	65	69	67	59	M	L
16	84	84	67	29	M	M
Control I	86	91	1	1	o	o
17	75	83	32	20	L+	L
18	75	85	76	52	H—	M+
19	97	88	17	5	L	L—
20	69	86	85	86	M	M
21	78	82	12	8	T	T
22	93	93	27	14	L	L
23	79	79	53	44	M	L+
24	81	70	36	35	L	T+
25	80	81	17	17	T+	L—
26	66	77	42	40	T+	T+
27	87	95	30	17	L+	L—
28	90	87	34	26	L	L—
29	90	88	25	13	L	L
30	87	90	9	3	L—	L—
31	83	85	23	29	L+	L
32	82	84	39	22	L+	L+
33	87	88	63	63	M	M
34	80	88	69	60	H—	H—
35	81	90	50	34	M—	L+
36	77	87	69	69	H	H
37	81	76	78	66	H+	H+
Control II	95	95	1	o	o	o

* Average of four pots.

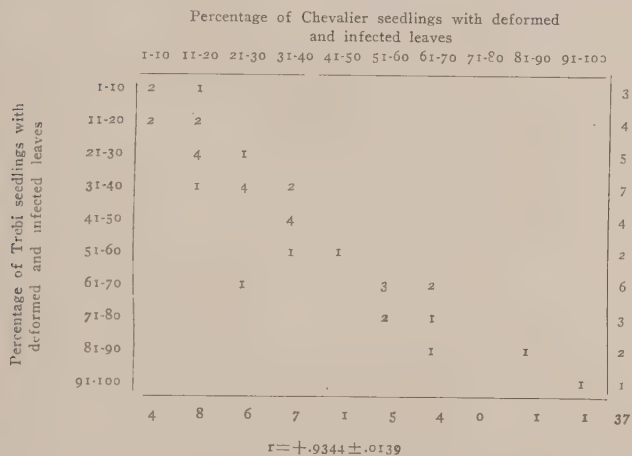† Due to primary infection, leaves often destroyed or contorted, and very much shortened.

‡ Gross appearances. H=heavy, M=medium, L=light, T=trace, o=no infection.

The degree of stunting on one-month-old seedlings, based on general appearance at that time, is also indicated. It will be seen that many of the physiologic forms differ in their reaction on two varieties of barley.

Forms 5, 11, 34, and 37 were especially virulent in both varieties. Two of these, Forms 5 and 11, were virulent on wheat also. Altho Forms 3, 4, 30, and others were weakly parasitic, they all attacked barley slightly. In general, the same forms were also weak pathogenes on wheat varieties.

The correlation coefficients for the reactions of 37 forms on two varieties of barley were computed. The data are presented in Table XIII. The results show a correlation of $+0.9344 \pm 0.0139$. This indicates that there is a high degree of correlation between the behavior of the forms on one variety and that on the other.

TABLE XIII

CORRELATION BETWEEN PERCENTAGE OF SEEDLINGS OF TREBI AND CHEVALIER BARLEY WITH DEFORMED AND INFECTED PRIMARY LEAVES RESULTING FROM INOCULATING THE SOIL WITH 37 PHYSIOLOGIC FORMS OF *H. sativum*

	Percentage of Chevalier seedlings with deformed and infected leaves										
	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	
1-10	2	1									3
11-20	2	2							·		4
21-30		4	1								5
31-40		1	4	2							7
41-50				4							4
51-60				1	1						2
61-70			1			3	2				6
71-80						2	1				3
81-90							1		1		2
91-100										1	1
	4	8	6	7	1	5	4	0	1	1	37

Percentage of Trebi seedlings with deformed and infected leaves

$r = +.9344 \pm .0139$

SPIKE INOCULATIONS

In order to determine whether physiologic forms differed in their ability to attack above-ground parts, individual heads of Marquis wheat and a selection of barley, Colsess (obtained from Prof. W. T. G. Wiener, of the Manitoba Agricultural College), were inoculated in the field with 28 forms of the pathogene. Four partly emerged heads of wheat and 3 of barley were inoculated with each form by dipping the heads in a spore suspension. After inoculation the heads were covered with glassine bags which were left on the wheat plants for 2 weeks and on the barley for 3 weeks. About 1 cc. of water was injected into the bags one week after inoculation. Similar tests were made in the greenhouse, except that pieces of cotton moistened in the spore suspension were wrapped around the heads and then covered with glassine bags.

The results of these experiments are summarized in Table XIV. All forms used infected both wheat and barley. Thus, it is apparent that blighting of wheat and barley florets, glumes, seeds, and spikes is caused by numerous physiologic forms of *H. sativum* (Plate II, Figs. 1 and 2). In many cases the upper sheath and neck of the inoculated barley plants became severely infected. This undoubtedly happened because some of the inoculum passed down between the sheath and stem, an ideal place for infection owing to the retention of moisture. The low percentage of infection on wheats in the field may have been due to unfavorable conditions during incubation, since a considerably higher degree of infection appeared on the same varieties in the greenhouse.

TABLE XIV

RESULTS OF INOCULATING HEADS OF WHEAT AND BARLEY IN THE FIELD AND GREENHOUSE WITH VARIOUS PHYSIOLOGIC FORMS OF *H. sativum*

Form No.	Field						Greenhouse		
	Barley					Wheat	Stage of development		
	Spike infection* Head No.				Neck and upper sheath infection†	No. of spikelets blighted‡	Barley	Wheat	
	1	2	3	Av.			Milk	Flower	Soft dough
Control	o	T—	o	T—	o	Tr—	o	o	o
1	H+	H+	H	H+	H+	5	. . .	H	T
2	L	M	. . .	L+	L	1	. . .	. . .	. . .
3	T	L	T	T+	T+	.5	. . .	. . .	. . .
5	H—	M+	H	H—	H—	1	H	L+	T
6	L	M	L+	L+	M+	8	. . .	. . .	- . . .
7	H—	M	M—	M	M+	1	. . .	o	o
8	M	L	M	M—	L+	2	M+	M—	T+
9	H+	H	H+	H+	H+	5	H—	H	H—
Control	o	o	o	o	o	1	o	o	o
11	H+	H+	M—	H	H+	3	. . .	. . .	. . .
12	H+	H	H	H	H	5	. . .	. . .	. . .
13	. . .	. . .	. . .	. . .	. . .	3	M—	M	L
14	M	H	L+	M	H	3	. . .	. . .	. . .
15	H+	H	H	H	H	2	. . .	. . .	. . .
16	H+	H	H	H	H	6	M—	L—	T—
17	M	H	M+	H	H	2	M	H+	H—
18	H+	H	H+	H+	H+	3	H	H—	L+
20	M	L+	L	L+	M+	1	M—	L+	T+
21	H	M	M	M+	L—	7	. . .	. . .	. . .
Control	T	o	T—	T—	o	. .	. . .	. . .	. . .
22	H—	M—	H—	M+	H—	2	. . .	. . .	. . .
23	M	L+	L	L+	M—	o	. . .	. . .	. . .
24	H	H	H	H	H	5	. . .	. . .	. . .
25	M	L	L—	L	M—	2	. . .	. . .	. . .
26	H	L	M	M	M	1	. . .	. . .	. . .
27	H+	H+	H+	H+	H+	5	. . .	. . .	. . .
31	L—	M+	H—	M	H	2	. . .	. . .	. . .
34	H+	H+	M+	H	H+	. .	. .	. . .	. . .
37	H+	H+	H+	H+	H+	8	. . .	. . .	. . .

* Symbols indicating degree of infection:
  H+ = all spikelets infected.
  H = over 80% spikelets infected.
  M = 40-80% spikelets infected.
  L = 10-40% spikelets infected.
  T = 1-10% spikelets infected.

† Neck and upper sheath of plants only inoculated.

‡ Average number of spikelets infected on four heads.

The 28 forms of *H. sativum* produced a brown to blackish discoloration on the glumes of wheat and barley. All caused embryo discoloration and local to general necrosis of the seed. In many cases the ovaries of the inoculated plants of both wheat and barley failed to develop. Barley spikes were frequently blighted completely in the flower or milk stage. In general, the controls remained free, or relatively free, from infection. In the latter cases the few diseased spikelets which developed were never so heavily infected as those which had been artificially inoculated. The organisms concerned were recovered repeatedly in pure culture from diseased spikelets of wheat and barley.

VARIATION IN PATHOGENICITY

From Table V it is evident that the degree of virulence is not correlated with the host or part of host from which the fungus was isolated. For instance, Forms 27 and 37 were both isolated from the kernels and Forms 2 and 3 from the leaves of barley, yet they show marked differences in pathogenicity on barley. Form 5 was isolated from a node of wheat and is one of the most virulent root-rotting forms of both wheat and barley.

By consulting Tables XI, XII, and XIV, profound differences are noted in the pathogenicity, not only of forms obtained from widely separated localities, but also between forms occurring in the same locality. Eleven distinct forms differing greatly in pathogenicity on wheat and barley were isolated from plants grown on a small plot of land at University Farm, St. Paul, Minn.

COMPARATIVE VIRULENCE OF *H. sativum* AND OTHER ROOT-ROTTING ORGANISMS

Comparative tests on the pathogenicity of several root-rotting organisms were made on Marquis and Mindum wheats in the greenhouse. The results are given in Table XV. They were obtained in a manner similar to that described for the experiment summarized in Table XI. It is obvious that one can either prove or disprove the assertion that *H. sativum* is a more virulent root parasite than *Gibberella saubinetii* (Durieu and Mont.) Sacc., or *Fusarium spp.* (Table XV). For example, if *G. saubinetii* is compared with *H. sativum*, Form 1, the difference in virulence is negligible, but *Gibberella* is much more virulent than Form 21. However, the opposite appears to be true if *Gibberella* is compared with Form 8 (Plate VI). No general statement can yet be considered final on this subject, because strains of *G. saubinetii* apparently also vary greatly in their degree of virulence; and many different *Fusaria* attack the roots of cereals. Furthermore, one must know the behavior of these organisms under various ecological conditions before any general conclusion can be made regarding their relative degrees of virulence.

TABLE XV

RESULTS OF GROWING MARQUIS AND MINDUM WHEATS IN SOIL INOCULATED WITH
DIFFERENT FUNGI

Organism	Percentage of seeds which produced seedlings										No. of plants which survived	
	Marquis					Mindum						
	Pot No.					Pot No.						
	1	2	3	4	Av.	1	2	3	4	Av.	Marquis	Mindum
Control	88	84	80	92	86	88	84	80	80	83	86	83
Gibberella saubinetii	48	44	40	52	46	44	40	24	28	34	19	6
Fusarium sp. (from corn)..	24	36	16	32	27	24	32	12	4	18	24	16
Fusarium moniliforme	80	68	76	88	76	68	64	68	68	67	76	67
H. sativum (Form 8).....	8	0	0	4	3	0	0	8	0	2	2	2
H. sativum (Form 1).....	20	28	28	24	25	16	20	24	36	24	4	10
H. sativum (Form 21)....	36	40	60	56	48	76	52	52	72	64	45	59

Our knowledge concerning the relative parasitic activities of these root-rotting organisms is limited to a few known strains or forms, and does not extend to the groups as a whole. Moreover, varietal resistance is a significant factor and must be taken into consideration.

STABILITY OF THE PHYSIOLOGIC FORMS

The range of variability of *H. sativum* is very wide. The character and rate of growth, the ability to reproduce, and the morphology of the organism are influenced profoundly by the kind, proportion, and amount of food available, and by other environmental conditions. Dosdall and Christensen (25), Stevens (62), and others have demonstrated this conclusively. Besides, it has been shown that there are many forms of *H. sativum* which respond differently to various environmental conditions (18 and 19).

Temporary phenotypes, or ecads, induced by differences in environmental conditions, occur frequently, altho the genotype remains unaltered. Such variants revert to the parental phenotype as soon as the causal stimulus is removed. Comparative cultural tests carried on for several years indicate that some forms of *H. sativum* remain constant and always appear the same under identical conditions. The question then arises: How did the forms originate, and are they relatively stable? New forms of life are generally supposed to arise as a result of hybridization or mutation.

POSSIBILITY OF HYBRIDIZATION

Hybridization is, of course, common in higher plants. There is some evidence that it also occurs quite frequently in fungi. Hybridization may take place at two different stages in the life cycle of fungi: (1) by the fusion of plus and minus strains, (2) by the anastomosing of vegetative hyphae.

Plus and minus strains occur in all four classes of fungi (6, 15, 21, 23, and 28). According to Blakeslee (6), Saito and Naganiski reported true hybrid zygospores between different species of *Mucor;* and Burgeff crossed a distinct mutant from a *Phycomyces sp.* with the original stock. Blakeslee (6) secured zygospore formation between many similar strains of *Mucor,* and between some which were quite different. The same author (6) observed what he terms "imperfect hybridization" between opposite sexes of different species of *Mucor,* and between sexes belonging to different genera.

Dodge (23) has demonstrated the presence of sexual strains in the genus *Ascobolus.* Buller (15) believes that there are at least four sexes in some of the *Hymenomycetes.* Blakeslee (6) found in all the hermaphroditic species of Mucorales investigated that all the spores in a "germ sporangium" were hermaphroditic. In the dioecious species there were two types of zygospore germination. The spores in the germ sporangium of *Mucor mucedo* were all of the same sex—plus or minus, never mixed. In the genus *Phycomyces* the germ sporangium may contain spores of both sexes. Thus, in *Mucor mucedo* sexual differentiation occurs when or before the zygospores germinate; in *Phycomyces,* in the germ sporangium. He states that induced growths from a germ tube of a sporangium in *Phycomyces* gave rise temporarily to a hermaphroditic plant. Such a plant differed strikingly from the normal plus and minus parent plants. According to Blakeslee (6), Burgeff has produced hermaphroditic strains of *Phycomyces* by mechanically mixing the protoplasm of plus and minus vegetative filaments.

Brierley (11) pointed out the possibility of genetic contamination in fungi by the fusion of hyphal cells without the intervention of the sexual stage. Stevens (62), Dosdall (24), Ocfemia (52), and Drechsler (26) have observed the anastomosing of germ tubes and hyphae of various species of *Helminthosporium.* The writer has observed the lateral fusion of as many as seven germ tubes of *H. sativum* in one series.

Matsumota (48) has observed fusion between the hyphae of different strains of *Rhizoctonia solani* Kühn. Brierley (11) recorded similar observations for *Botrytis cinerea* Pers. Ezekiel (29) observed that hyphal anastomosis took place in agar plates at the junction of colonies of similar strains of Sclerotinia, as well as between different species of this genus.

However, colonies of different forms of *H. sativum,* and even of the same form, usually do not fuse, because they are antibiotic to each other (Plate I, Fig. 2). Many plates were inoculated with different species of *Helminthosporium,* and with distinct forms of *H. sativum;* but, in every case only the parental types appeared. However, too

much importance should not be attached to this preliminary test. Each cell contains a variable number of nuclei from one to four (62), and there are therefore many chances for normal or abnormal rearrangement of chromosomes or complete nuclei.

MUTATION

Recent publications (7, 9, and 62) indicate that asexual mutations in fungi occur frequently on culture media. Mutations have been reported for fungi belonging to the *Phycomycetes, Ascomycetes,* and *Fungi imperfecti.* In 1922, Stevens (62) presented evidence that mutations were common in *Helminthosporium* growing in culture. In the present studies, much of Stevens' work was repeated and extended; and, in general, the results were corroborated.

Numerous and widely different types of mutants, usually occurring in sectors, have been observed repeatedly. These variations arose from parts of mycelium and were generally wedge-shaped or fan-shaped (Plates VII and VIII). Under certain conditions mass-like mutation apparently occurred in some forms. This type of mutation has recently been described in higher plants also (12).

In the present work numerous transfers were made from sectors and normal parent material. When the original variant was chosen from a sharply defined sector of a relatively young culture, as illustrated in Plate VII, it always developed into a colony distinct from its parent. Furthermore, a study of subsequent transfers from these sectors indicated that the changes were genotypic and not mere changes in phenotypes due to environmental conditions. Single-spore isolations, whether from the sector in which a variant originated, or from colonies developed from mass transfers, grew similarly on culture media. In either case the colonies were all alike. Mutants bred true when propagated from spores or mycelium. Stevens (62), Burger (16), and others also have demonstrated that spores from mutants again produced the mutant type.

In order to ascertain whether there were any differences in the tendency of various forms to mutate, two kinds of media were inoculated. Each form was grown in triplicate plates of uniform size, containing 18 cc. of the medium. The plates for each series were inoculated on the same date, and kept on the same table in the laboratory. Notes on the number of sectors were taken on the 11th and 15th days after inoculation. As time would not permit the culturing and studying in detail of all these variations, the term sector is often used in the following discussion whenever the corresponding breeding test was not made. Table XVI summarizes the results of the experiment.

TABLE XVI

NUMBER OF SECTORS, OR APPARENT MUTATIONS PRODUCED BY 37 PHYSIOLOGIC FORMS OF *H. sativum* ON POTATO DEXTROSE AGAR AND OATMEAL-RICE-CORNMEAL AGAR

Form No.	Potato dextrose agar				Oatmeal-rice-cornmeal agar			
	Age, 11 days		Age, 15 days		Age, 11 days		Age, 15 days	
	No. of plates with sectors	Total No. of sectors	No. of plates with sectors	Total No. of sectors	No. of plates with sectors	Total No. of sectors	No. of plates with sectors	Total No. of sectors
1	I	I	2	3	I	I	I	2
2	0	0	0	0	2	3	2	5
3	3	5	3	8	2	3	3	8
4	0	0	3	3	0	0	2	5
5	0	0	0	0	0	0	2	6
6	2	7	2	8	0	0	2	3
7	0	0	0	0	0	0	0	0
8	I	3	I	4	I	I	3	3
9	2	2	2	3	3	5	3	16
10	0	0	0	0	I	I	2	2
11	I	I	I	I	2	2	2	2
12	0	0	0	0	0	0	0	0
13	0	0	0	0	I	I	I	I
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	I	I	I	I	3	8	3	8
17	I	I	I	2	3	7	3	9
18	0	0	0	0	I	I	I	2
19	0	0	0	0	I	I	2	4
20	I	I	2	2	3	10	3	15
21	0	0	0	0	0	0	0	0
22	I	3	2	8	0	0	I	I
23	0	0	0	0	0	0	0	0
24	0	0	0	0	I	I	I	I
25	0	0	0	0	I	I	I	I
26	2	2	2	2	0	0	0	0
27	0	0	0	0	0	0	0	0
28	I	I	I	I	2	2	2	3
29	I	3	I	3	0	0	0	0
30	2	5	2	5	3	5	3	13
31	0	0	0	0	3	7	3	8
32	0	0	0	0	0	0	2	4
33	0	0	0	0	3	19	3	36
34	2	2	2	4	3	3	3	3
35	0	0	0	0	I	4	I	4
36	2	3	2	3	3	8	3	15
37	I	I	I	I	0	0	0	0

It is evident that some forms mutate more often than others. Mutations occurred in forms obtained from England, Australia, Africa, Argentina, Serbia, and Canada and from various localities in the United States.

The data in Table XVI also show that a given form may mutate more frequently on one nutrient medium than on another. On potato dextrose agar 18 out of 37 forms developed sectors, while on oatmeal-rice-cornmeal agar sectors occurred in cultures of 27 of the 37 forms. Seven forms failed to develop sectors on either medium. Forms 26, 29, and 37 gave rise to apparent mutations on potato dextrose agar,

but not on the other medium. Twelve of the forms which did not mutate on potato dextrose agar did so on the oatmeal-rice-cornmeal agar. The difference in the number of sectors of certain forms on the two media sometimes was very marked. Thus, Form 33 developed 36; Form 30, 15; Form 16, 8 sectors on oatmeal-rice-cornmeal agar; but none, 5, and 1, respectively on potato dextrose agar.

Some of these mutants have been grown on several different media for varying lengths of time, but they have remained constant for the characters under observation. Mutants appeared as stable as the various forms of *H. sativum* which have been isolated from host tissue. Altho it is true that several mutants apparently reverted, these reversions were always in the form of a sector. This agrees with the observations of Stevens (62). Temporary modifications are not considered here. They sometimes appear, especially in old cultures, but they revert to the parental type on the first transfer.

Some of the mutants which were cultured mutated still further. Colonies of a mutant have been observed to produce distinct sectors which in turn mutated while still in the same plate. Two of the mutants gave rise constantly to new mutants, their behavior suggesting that they might be segregating heterozygotes.

Roberts (54) observed two variants, "A" and "B", in the progeny of a single conidium of *Alternaria mali* Roberts derived from a succession of 15 singly selected conidia. For the first 10 selections, variant "A" had a slight tendency to break up into "A" and "B"; but from then on it remained constant. Variant "B" broke up into "A" and "B" sectors for 57 selections, but thenceforward it grew true to type. Stevens (62) observed that a number of *Helminthosporium* mutants were unstable and constantly gave rise to new variants.

The mutants, like the 37 forms of *H. sativum* previously mentioned, behave differently on different culture media. For instance, on a given medium a mutant sometimes resembles its parent very closely, but on a different medium it sometimes is so different culturally as to look like another species.

Altho Form 1 had been grown on hundreds of plates from the time it was isolated in the winter of 1919, no distinct sectors were ever observed until the winter of 1924-25. On the other hand, Form 3 was constantly mutating, especially on green bean agar. While determining the effect of various amounts of nutrient media on zonation, it was repeatedly observed that colonies of Form 1 growing in petri dishes containing 10 cc. and 15 cc. of oatmeal-rice-cornmeal agar produced numerous sectors. At the same time and under similar conditions, none appeared in dishes containing 20 and 30 cc. of medium. The history of Form 1 is shown diagrammatically in Figure 1.

Colonies which gave rise to mutations in the petri dishes marked I to II in Figure I were all started from mass transfers. Twenty single-spore isolations were made from the same tube. Ten of the spores were used in inoculating 10 plates, each containing 30 cc. of green bean agar, and 10 for the same number of oatmeal-rice-cornmeal agar. Not a single sector appeared in the 20 plates inoculated with single spores; neither did any occur in the two controls. The 10 colonies on each medium derived from single spores, and the control, were all alike in general appearance in each case (Plate IX). This indicated the homozygosity of this form. La Rue (41) concluded that selection is totally ineffective in establishing distinct lines within a pure strain of *Pestalozzia guepini* Desm. However, in order to obtain additional evidence regarding variations by single-spore selection, the following experiment was made (Fig. 1).
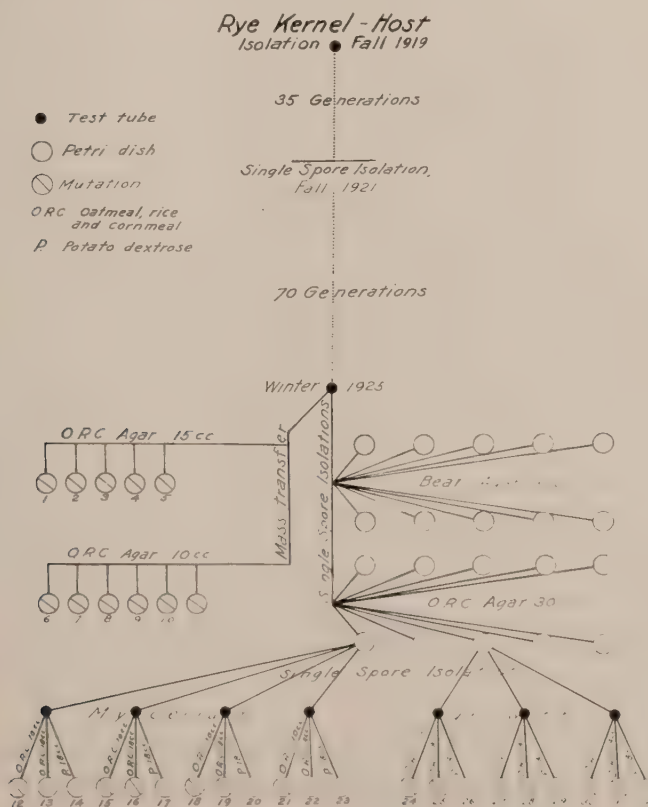

Fig. 1. Graphic Representation of the History of *H. sativum*, Form 1

Three and four single-spore isolations were made from each of two monosporous cultures which were previously derived from a culture of single-spore origin. The conidia were transferred to test tubes, and mycelium only had developed at the end of 4 days. From each of these tubes 3 plates were inoculated, one containing 18 cc. of potato dextrose, one 18 cc. of oatmeal-rice-cornmeal agar, and one 10 cc. of the latter medium. From Figure 1 it is apparent that mutations appeared in all plates with 10 cc. of agar, and in 4 of the 7 plates which contained 18 cc. of the combination agar. None whatever arose in the 7 plates containing potato dextrose agar.

In order to determine the effect of the amount of medium on the expression of variation, the following test was made. Newly poured agar plates were placed on a slight incline so that the upper side was covered with a thin layer of the medium, the lower by a considerably thicker layer. These slants were inoculated in the center in the usual manner. Numerous sectors developed, but only in the region of the thinner layer of agar (Plate VII, Fig 2). That some of these sectors were true mutants was proved by successive transfers.

Table XVI indicates that mutations may occur at different distances from the source of inoculation (Plates VII and VIII). Sectors have been observed to arise from near the center of the colony but, for the most part, the number of variants was greater near the edge.

Blakeslee (7) obtained mutants from a hermaphroditic fungus, *Mucor genevensis*, which apparently had their origin within the spore, or at least within the sporangium. In the present investigation there was no evidence that the differentiation which gave rise to mutation occurred in the spore. It will be seen from Figure 1 that colonies 1 to 11, obtained from mass inoculation, gave rise to 36 mutations. Colonies 12, 15, 18, 21, 24, 27, and 30 arose directly from the mycelial growth of a single spore, which in turn was derived from three successive single-spore transfers. They developed 38 apparent mutants. These results and similar results with Forms 22 and 24 indicate that mutations occur as frequently in cultures derived from single conidia as from colonies resulting from mass transfer of spores and mycelium.

The mutants studied varied from the parents in the following characters: (1) rate of growth, (2) nature of mycelial growth and whether cottony or felty, etc., (3) conidial production, (4) conidial clusters, (5) color, and (6) zonation. Many of the gross characters in some forms were quite different from any of those observed in strains or forms isolated from host plants. These mutants differed culturally from each other quite as much as do certain species of *Helminthosporium* — *H. teres* Sacc., *H. pedicellatum* Henry, and *H. gramineum* Rab. In general the variations observed were similar to those reported by Stevens (62).

Apparently most mutations in fungi have been due to the loss of a factor or group of factors for color, and of a factor for abundant fructification. Stevens (62) states that mutants with low conidial production, verging on sterility, coupled with paleness of colony, occurred with the greatest frequency. This is in accord with the writer's findings.

Several distinct types of mutants frequently occurred in the same plate, but usually a given form gave rise to one or two types only. A single colony has given rise to twelve similar mutants. Within a period of 6 months, however, Form 1 has thrown as many as seven distinct mutants which retained their distinctive characters after repeated transfers.

Just how these mutations arose is not known. They may have arisen through what might be termed normal nuclear rearrangement, or through aberrant chromosomal distribution, or gene changes. It has been impossible to determine accurately the genetic constitution of the parents and their mutants, because the nuclei and chromosomes are very small and the plants are propagated asexually, thus rendering cross-breeding experiments futile. Even if a variant resulted from the fusion of two adjoining cells, it might not necessarily be considered a normal process of combination or segregation, as it has been shown that anastomosing of hyphae is an extremely common phenomenon in *H. sativum*.

From Figure 1 it is apparent that mutations were not observed in *H. sativum*, Form 1, until after it had grown in culture for 6 years, during which time it went through more than 100 "generations." Form 3, on the other hand, from the time it was isolated four years ago, has had a conspicuous tendency to mutate, especially on green bean agar. The sexual stage of *H. sativum* has never been reported. Many and varied attempts to produce it in culture have given only negative results. Apparently the recent intervention of a sexual stage is not essential for mutation in those fungi which can propagate sexually.

Several investigators have tried to induce mutation by environmental changes. According to Brierley (10 and 11), Arcichovskij, Waterman, and Schiemann have described mutation in *Aspergillus niger* and *Penicillium glaucum*. These authors claim to have induced mutation in these fungi by various environmental changes, such as the addition of zinc sulphate to the substrate, exposure to high temperatures, etc. Brierley (10) repeated much of their work but could not confirm it.

Stevens (62) unsuccessfully attempted to produce "saltations" by artificially wounding, by mixed planting, and by implanting.

PATHOGENICITY OF MUTANTS COMPARED WITH THAT OF THEIR PARENTS

In order to ascertain whether there are any differences between the degrees of virulence of mutants and their parents, comparative tests were made on Trebi barley and Marquis wheat. The experiment was similar to that already described for testing the pathogenicity of the 37 forms of *H. sativum* on barley, except that six pots of each cereal were used. The virulence of 13 mutants was compared to that of their parents. Notes were taken on the percentage of plants that emerged, on the percentage of plants with deformed primary leaves, and on the degree of stunting. The results are presented in Table XVII.

TABLE XVII

RESULTS OF INOCULATING SOIL GROWING TREBI BARLEY AND MARQUIS WHEAT, WITH 4 PHYSIOLOGIC FORMS AND MUTANTS OF *H. sativum*

	Wheat			Barley		
	Percentage			Percentage		
Organisms	Seeds developing into seedlings*	Infected and deformed primary leaves	Virulence in comparison to parent†	Seeds developing into seedlings	Infected and deformed primary leaves	Virulence in comparsion to parent
Form 1						
Parent	76	22.7	....	88	17.3	....
Parent S₈‡	73	21.9	Same	86	23.3	Same
Mutant 1	76	35.0	Greater	81	72.3	Greater
" 3	86	15.5	Same	89	11.2	Same
.. 4	85	7.8	do	81	9.8	do
" 5	83	23.2	do	88	19.0	do
" 6	78	23.0	do	84	23.0	do
.. 21	90	0.0	Less	84	3.5	Less
" 22	90	3.7	do	88	4.5	do
" 25	73	23.2	Same	86	24.0	Same
Form 8						
Parent	28	85.7	....	70	90.0	....
Mutant 75	22	84.5	Same	68	98.0	Same
Form 22						
Parent	76	23.0	....	81	13.6	....
Mutant 40	31	85.0	Decidedly greater	72	91.6	Decidedly greater
" 41	84	10.0	Same	88	6.1	Same
Form 24						
Parent	72	26.0	....	86	22.1	....
Mutant 30	76	23.7	Same	84	26.0	Same
" 31	88	7.4	Less	81	5.7	Less

* Based on six pots of 25 seeds each.

† Based on percentage of plants that emerged, on percentage with deformed and infected primary leaves, and on general degree of stunting.

‡ Derived from three successive single-spore isolations.

The mutants did not all possess the same degree of virulence. Most of them were like the parent form, but two were decidedly more virulent than their respective parents. Mutant No. 40 was outstandingly more virulent than its parent, Form 22, on both barley and wheat (Plate X, Figs. 1 and 2). Three of the mutants and possibly more were less virulent than their parents. A decrease in virulence is harder

to detect than a change in the opposite direction, when the parents are relatively weak forms.

Mutant 40 was derived from a single spore from a monosporous culture of Form 22. It arose as a distinct sector, similar to the one shown in Plate VII, and had been transferred several times and grown on three different nutrient media. The cultural characteristics were not only different from those of its parents, but were unlike those of any of the forms under observation.

This mutation in virulence probably is going on continually in nature. It complicates the problem of controlling the disease and makes extreme caution necessary in drawing conclusions regarding the virulence of the pathogene. It probably means also that resistant varieties may be resistant only for a certain length of time and for certain localities. The duration of usefulness of resistant varieties probably is largely a matter of chance. Unusually virulent forms may arise by mutation. In this case the resistant varieties may become susceptible. On the other hand, it should be remembered that some of the mutants are less virulent than the parents. It is even possible that these weakly parasitic mutants might be eliminated in the struggle for existence.

It is especially interesting to note that physiologic forms of all pathogenic fungi do not necessarily behave alike. The physiologic forms of the stem rust fungi appear to be quite stable. There is considerable evidence that they do not mutate commonly, and that hybridization, if it occurs at all, is relatively infrequent (53, 58, and 59). But the physiologic forms of *H. sativum* are quite different in this respect. Some of them are very stable while others are extremely unstable. It is obvious that one can not generalize too much about physiologic specialization. General statements about the pathogenicity of a pathogene mean little without a knowledge of the number, distribution, and parasitic capabilities of different physiologic forms.

FORMATION AND GERMINATION OF SPORES
EFFECT OF ENVIRONMENTAL FACTORS ON FRUCTIFICATION

During the last four years the writer has made numerous attempts to induce *H. sativum* to produce an ascigerous stage. Infected seed of wheat and barley, heads of wheat, seedlings and cultures of the fungus were placed outdoors during the winter. Some of this material was buried in the ground at different depths; some was left in the pots in which it was grown. No perithecia developed. Cultures of the organisms were grown on different nutrient media and subjected to different conditions of temperature, moisture, and light, but only the

conidial stage developed. Starvation and the addition of toxic compounds proved fruitless. The pathogene was cultured on more than 100 different culture media and plant tissues, but all attempts to induce perithecial formation were unsuccessful. More than 70 strains, including many physiologic forms, were grown for years on culture media and on wheat heads, but no indication of the sexual stage was ever observed. Many mixed cultures of different physiologic forms were grown on wheat heads and on different nutrient agars, but they produced asexual spores only.

Many forms of *H. sativum* produced conidia freely on various plant tissues and on artificial media, while others apparently seldom fructified on culture media. Chlamydospore-like structures formed frequently within a liquid substrate. An abundance of spores was produced on a 2 per cent sucrose solution. The organism also fructified on diseased plant parts beneath the surface of the sandy soil. On sterilized soil to which organic material was added, certain forms sporulated profusely, and others grew well but developed no spores. In some seasons the pathogene sporulated freely on the above-ground parts of the host plants.

The results, showing the effect of light and temperature on the production of conidia by Form 1, are summarized in Table XVIII. It seems safe to conclude that the production of conidia is influenced greatly by temperature, but not appreciably by light.

TABLE XVIII

Relation of Temperature and Light to Formation of Conidia by *H. sativum*, Form 1, on Potato Dextrose Agar

Temperature, degrees C.	Light relation	Days required for fructification	Conidial production
5-7	Dark	?	None
10-12	do	10	Trace to light
16-17	do	3	Abundant
20-21	do	3	do
20-21	Alternation (lamplight)	3	do
20-30 (changed every 12 hrs.)	Dark	3	do
24-25	do	3	do
29-30	do	3	Trace
32-33	do	?	None
Outdoors	Diurnal changes	3	Abundant
Greenhouse	do	3	do
Buried in soil	Dark	?	do
20-22	Continuous light	?	do

The optimum temperature for the production of conidia by Form 1 seems to be between 16 and 25° C. Changes within these limits have little effect. The maximum appears to be about 29° C. At any rate, temperatures of 29° C. or higher partly inhibit spore formation. The minimum temperature at which spores are formed seems to be from

10 to 12° C. Only a few were produced at this temperature, and none were produced at a temperature of from 5° to 7° C. Here again, however, it is important to remember that the different physiologic forms may react differently to temperature. In fact, there is direct evidence of this fact. Dosdall (24) observed that *H. sativum*, strain 82A (the writer's Form 2), sporulated well at 32° C. At this temperature Form 1 grew scarcely at all, and produced no spores.

The time required for the production of spores varies somewhat with the conditions. On a few occasions conidia germinated and produced spores within 24 hours. Conidia were produced within this time from spores sown in syracuse dishes containing 0.5 cc. of water and bits of host tissue. Usually, however, 48 hours or longer was required. Mycelium on diseased tissue sporulated in from 12 to 24 hours. Unboiled polished rice, kept moist, was covered with a dense mass of spores 3 days after it had been inoculated with conidia of *H. sativum*.

The same mycelium may produce more than one crop of spores. This was demonstrated by growing the organism on a solid substratum and scraping off the entire crop of conidia after the mycelium had sporulated abundantly. Within 3 or 4 days a new crop of conidia had been produced. It is quite probable that several successive crops of spores are produced in nature. The fact that abundant conidia can be formed within a comparatively short time is very significant in evaluating the parasitic capabilities of the fungus.

EXPERIMENTS ON ATTENUATION

Attenuation of virulence of fungi after continued culturing on artificial media has been reported occasionally. Mitra (49) stated that *H. turcicum* Pass. lost its parasitic nature if cultivated for a long time in culture. Inoculation with a strain kept in culture a year failed to produce infection. Krakover (40) reported attenuation in virulence for *Macrosporium sarcinaeformis* Cav. from red clover. Bonar (9) stated that long continued growth on culture media of *Brachysporium trifolii* Kauff. caused an attenuation of its virulence.

Six years of culturing on artificial media, mostly on potato dextrose agar, did not change the virulence of *H. sativum*, Form 1. After 3 years of dormancy, Form 4 was again transferred to culture media. Its pathogenic capabilities were not altered. Most of the virulent forms of *H. sativum* here reported have been cultured for 3 or 4 years on various cultural media. Even if a change in pathogenicity should occur over several years, it could more plausibly be explained by mutation than by attenuation due to culturing. Stevens (62) reported apparent mutation of *Helminthosporium spp.* in test-tube cultures. The writer

obtained some evidence that mutation occurred also in test-tube cultures of *H. sativum*, notably in Forms 7 and 23. Single-spore isolations from these two forms, developed on potato dextrose agar, did not sporulate when sown on the same medium.

SPORE GERMINATION

The process of spore germination in *H. sativum* and some of the factors influencing it have been described by Dosdall (24). She found that spores of culture "82 A" (Form 2) germinated in redistilled water equally well at temperatures ranging from 6° to 39° C. The optimum, based on length of germ tube and time required for emerging, was between 22° and 30° C. Spores germinated at hydrogen-ion concentrations of pH 2.4 to pH 12. These facts show that the conidia germinate under a wide range of conditions.

However, the results obtained by the writer indicate that spore germination may be influenced profoundly by slight changes in environment. Relatively few spores germinated in the early tests, hence a study was made to determine the most important factors affecting germination.

EFFECT OF PLANT TISSUES

Several investigators have observed that the presence of host tissue affects spore germination of various pathogenes. Anderson (2) showed it for pycnidiospores of *Endothia parasitica* (Mur.) Anders.; Brown (13) for conidia of *Botrytis cinerea* Pers.; Leach (42) for spores of *Colletotrichum lindemuthianum* (Sacc. and Mag.) Bri. and Cav.; Noble (51) for spores of *Urocystis tritici* Koern.; and Whitehead (67) for spores of *Urocystis cepulae* Frost.

The effect of host tissue on spore germination of *H. sativum* was tested by placing conidia in syracuse dishes containing from 0.5 to 1.0 cc. of distilled water. To these dishes from 10 to 20 small pieces (0.5 to 1.0 cm. long) of barley seedlings were added. The controls contained distilled water only. All tests were made in duplicate, usually at room temperature, and the percentage of germination was calculated from a count of 100 spores in each dish. In every case, unless otherwise indicated, spores of Form 1 grown on sterilized wheat heads were used. Some of them had been stored for varying periods of time outdoors or in the laboratory. Those which were kept outside had been wrapped in small cheesecloth packets and buried just below the surface of the ground.

The spores germinated best and most uniformly in the dishes containing host tissue (Table XIX). The greater fluctuation in the series from outdoors was probably due to the presence of some toxic substance in the sand or soil. Table XIX does not give an adequate idea of the stimulatory effect of plant tissue. Within 24 hours nearly all

spores germinating in dishes containing host tissue had developed long, very much branched, and vigorous germ tubes, often extending across the low-power field of the microscope. In sharpe contrast, spores germinating in containers without host tissue usually produced relatively weak, short, unbranched germ tubes. Furthermore, the spores in dishes containing host tissue almost always germinated from both ends, while those in the control dishes frequently germinated from one end only.

TABLE XIX

GERMINATION OF SPORES OF *H. sativum*, FORM I, IN DISTILLED WATER WITH AND WITHOUT THE ADDITION OF BARLEY LEAF TISSUE

Time of exposure, days	Percentage of germination											
	Laboratory						Outdoors					
	Distilled water			Distilled water with tissue			Distilled water			Distilled water with tissue		
	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.
125	22	25	23.5	96	94	95.0	4	1	2.5	98	90	94.0
155	36	76	56.0	97	98	97.5	0	1	0.5	96	95	95.5
186	95	77	86.0	99	99	99.0	82	87	84.5	99	98	98.5
209	30	10	20.0	99	99	99.0	1	0	0.5	90	70	80.0
242	..	..	...	..	..	...	82	94	88.0	94	96	95.0
310	70	65	67.5	96	98	97.0	11	7	9.0	83	96	89.5

It was observed also that in the controls there was a higher percentage of germination of spores floating on the surface than of those which were submerged. This is in accord with Dosdall's results (24). On the other hand, no differences could be detected in percentage of germination of submerged spores or those on the surface when host tissue was added. Preliminary tests indicated that newly-formed spores germinate more freely under water in the absence of host tissue than do more mature spores. There is some evidence, also, that the substratum on which the spores are produced affects the percentage of spores that germinate in distilled water. This may possibly account for the high percentage of germination observed by Dosdall (24). But, even under the most favorable conditions, the maximum germination in her experiments seldom exceeded 90 per cent. By the addition of host tissue, the writer consistently obtained from 95 to 100 per cent germination of spores which had been kept in the laboratory for varying periods of time.

Brown (14) found that certain plants produced volatile substances which stimulated spore germination of several fungi while substances from other plants were ineffective or actually deleterious. In his work on the germination of spores of *Colletotrichum lindemuthianum*, Leach (42) obtained some evidence that tissues of plants other than the host stimulated germination. Noble (51) showed definitely that

uninjured seedlings of non-susceptible plants (field peas, beans, rye) stimulated the germination of spores of *Urocystis tritici.*

The effect of various non-susceptible plant tissues on germination of conidia of *H. sativum,* Form 1, was determined (Table XX). Apparently the stimulatory agent is not specific. Wheat, oats, rye, bluegrass, strawberry, sunflower, tomato, and potato tuber tissues stimulated germination. But bits of very young potato sprouts inhibited the development of germ tubes, possibly because of the presence of the alkaloid solanin. Many of the spores in one syracuse dish containing tomato tissues failed to germinate normally, the germ tubes often emerging and then producing deformed branches. Leach (42) also noticed abnormal branching of the germ tubes of *C. lindemuthianum* when tissues of tomato and several other plants were added to the distilled water. Altho the percentage of germination of conidia of *H. sativum* was usually not reduced by the presence of tissues from non-susceptible plants, the vigor of the germ tubes was usually greater in the presence of tissues from susceptible plants.

TABLE XX

GERMINATION OF SPORES OF *H. sativum,* FORM 1, IN DISTILLED WATER, WITH AND WITHOUT THE ADDITION OF TISSUES OF VARIOUS PLANTS

Plant part	Percentage of germination			Degree of vigor of germ tubes
	Ser. A	Ser. B	Av.	
Barley (seedlings)	96	94	95.0	Very vigorous
Wheat do	97	95	96.0	do
Oats do	96	97	96.5	do
Rye do	94	94	94.0	do
Bluegrass (leaf)	98	96	97.0	Vigorous
Strawberry do	93	90	91.5	do
Sunflower do	92	97	94.5	do
Tomato do	97	94	95.5	Many very much distorted and failed to develop. Some tubes vigorous.
Potato tuber	94	96	95.0	Vigorous
Green potato shoots (1-2 cm. long)	65	83	74.0	Weak, less than 20 microns long and failed to develop
Control without tissue (spores 14 months old)	40	38	39.0	Weak
Without tissue (spores 1 month old)	46	55	50.5	do

Several tests were made in order to determine the effect of soil and manure extracts on spore germination (Table XXI). Only a few spores germinated in these extracts. In fact, they seemed to be toxic, but the addition of host tissue counteracted their toxic effects to a considerable extent. Boiling apparently destroyed the toxic substance. As *H. sativum* is a common saprophyte in the soil, it is surprising that the spores germinate so poorly in soil and manure decoctions.

TABLE XXI

GERMINATION OF SPORES OF *H. sativum*, FORM 1, IN SOIL AND MANURE EXTRACTS WITH AND
WITHOUT THE ADDITION OF HOST TISSUE

Material from which extract was made	Percentage of germination and vigor							
	Medium with host tissue				Medium without host tissue			
	Ser. A	Ser. B	Av.	Vigor	Ser. A	Ser. B	Av.	Vigor
Check, distilled water	99	99	99.0	Very vigorous	38	62	50.0	Fair
Boiled peat	91	98	94.5	Vigorous	13	24	18.5	Weak
Unboiled peat	70	97	83.5	do	19	15	17.0	do
Well-rotted manure..	96	88	92.0	Very vigorous	5	3	4.0	Very vigorous
Heavily manured soil	27	60	43.5	Weak	4	1	2.5	do
Boiled heavily ma-					80	50	65.0	Fair
nured soil	98	97	97.5	Very vigorous				
Clay soil	45	76	60.5	Fair	31	9	20.0	Weak
Boiled clay soil.....	96	95	95.5	Vigorous	8	16	12.0	do
Greenhouse soil	96	98	97.0	Very vigorous	29	23	26.0	do
Sterilized greenhouse					15	11	13.0	do
soil	98	98	98.0	do				
Standing water (out-					17	13	15.0	do
side	90	96	93.0	do				
Dirty snow water...	97	84	90.5	do	9	14	11.5	do

VITALITY OF CONIDIA

Other things being equal, any pathogene which produces large
numbers of spores under a fairly wide range of conditions is much more
likely to become widely disseminated and established than one which
produces spores only with difficulty and under a rather narrow range
of conditions.

But spore production alone does not insure the success of a patho-
gene. The spores must be disseminated and must be able to withstand
unfavorable conditions. Nor must they germinate too readily. If they
do, it is likely that they may often germinate when there is nothing
available for them to infect.

The results of the writer's studies on the vitality and germination
of spores indicate clearly that the pathogene is well fortified against
adversity. The spores can withstand low as well as high temperatures,
provided the relative humidity is favorable. Furthermore, they can
withstand long periods of alternate freezing and thawing. They can
even remain in water for a year or longer without germinating. When
host tissue is added to the water, however, the spores germinate
promptly. This suggests the possibility that the spores germinate most
readily in nature only in contact with host tissues.

The combined effect of various temperatures and relative humidities
on the viability of conidia was tested. Spores were stored at relative
humidities of 7.1, 30.7, 45.7, 73.4, 87.7, and 95 to 100 per cent at 6°,
12°, 24° and 29° C. The relative humidities, except the last one, were
maintained in wide-mouthed bottles of 100-cc. capacity containing 50 cc.
of saturated solutions of lithium chloride, sodium hydroxide, copper

nitrate, sodium chloride, and potassium sulphate, respectively, as suggested by Peterson (34). The 95 to 100 per cent humidity was obtained by using distilled water. Small paraffined cardboard baskets containing the spores were attached to the stoppers in such a manner than when the stoppers were replaced the baskets hung slightly above the surface of the liquids. The conidia used were grown on sterilized wheat heads in a single large Erlenmeyer flask. The corks were inserted tightly and covered with a coating of melted paraffin. Germination tests were made at the end of 82 days. The results are given in Table XXII.

TABLE XXII

EFFECTS OF TEMPERATURE AND HUMIDITY ON THE LONGEVITY OF CONIDIA
OF *H. sativum*, FORM 1*

Storage temperature	Percentage of relative humidity	Percentage of germination			Vigor
		Ser. A	Ser. B	Av.	
5°- 7° C.	7.0+	99	100	99.5	Very vigorous
"	30.0+	90	94	92.0	do
"	45.7	100	98	99.0	do
"	73.4	98	98	98.0	do
"	89.7	99	96	97.5	do
"	90.0+	99	100	99.5	do
10°-12° C.	7.0+	97	90	93.5	do
"	30.0+	88	89	88.5	do
"	45.7	84	81	82.5	do
"	73.4	98	93	95.5	do
"	89.7	98	99	98.5	do
"	90.0+	97	99	98.0	do
24°-25° C.	7.0+	100	94	96.0	do
"	30.0+	99	93	96.0	do
"	45.7	0†	0†	...	. . . .
"	73.4	100	98	99.0	Very vigorous
"	89.7	10‡	15	12.5	Fair
"	90.0+	30‡	34	32.0	do
29°-30° C.	7.0	91	93	92.0	Very vigorous
"	30.0	89	96	92.5	do
"	45.0	†	. .	. . .	. . . .
"	73.0	0	0	0	. . . .
"	90.0+	0	0	0	. . . .

* Experiment begun December 12, 1924.

† Spores discolored and killed by chemical in jar.

‡ Covered by growth of molds.

It is evident from Table XXII that humidity has a greater effect on viability than has temperature. Spores remained viable at low relative humidities (7 and 30 per cent) for a long time at the higher as well as at the lower temperatures. But at the higher temperatures high relative humidity was deleterious. At the higher temperatures toxic fumes were given off by the copper nitrate, so the results at a relative humidity of 45.7 per cent are not reliable.

The results of exposing spores and mycelium and seed infected with mycelium to high temperatures are presented in Table XXIII. Exposure for 10 minutes at 54 C. killed spores and mycelium in water, but

did not kill the mycelium in the seed. Similar results were obtained by soaking barley seeds in water at 52° C. for 15 minutes. The results in Table XXIII indicate also that a long-time immersion in water at 46° to 48° C. is more effective than a short immersion at 50° to 52° C. It is apparent that the standard hot-water treatments will not kill all of the mycelium in the seed of wheat and barley.

TABLE XXIII

RESISTANCE OF SPORES AND MYCELIUM OF *H. sativum*, FORM I, AND OF THE MYCELIUM IN INFECTED KERNELS OF PENTAD WHEAT TO HOT WATER AT THREE DIFFERENT TEMPERATURES

Treatment	Material treated		
	Spores	Mycelium	Infected wheat
46°-48° C. for 2 hours	Few spores germinated, germ tubes weak	Required 5 days incubation before growth became visible	4 colonies developed from 20 seeds
50°-52° C. for 17 minutes	About 5 per cent of spores germinated, germ tubes weak	Not killed	4 colonies developed from 20 seeds
54° C. for 10 minutes	No germination	No growth	2 colonies developed from 20 seeds
Control	99 per cent germination	Rapid growth	16 colonies developed from 20 seeds

Preliminary tests were made on the effect of alternately freezing and thawing spores submerged in water. During the winter, syracuse dishes containing from 1 to 2 cc. of a suspension of spores in water were placed outside on the north side of the building. From time to time the dishes were taken into the laboratory, and after the ice had melted they were again placed outside. Sometimes the rise or fall in temperature was as much as 40° C. in a few minutes. Germination tests were made at the end of 6 weeks. In one lot, 50 per cent of the spores germinated. Spores on moist wheat kernels subjected to similar conditions germinated profusely. Eventually, after several months of this treatment, the spores were killed, but it is clear that spores can withstand alternate freezing and thawing for considerable periods of time.

To test further the effect of exposure on the vitality of conidia, spore material was grown on sterilized wheat heads which were wrapped in pieces of cheesecloth containing a handful of white sand, and buried outdoors just beneath the surface of the soil. In 1923 tests were made from material kept at University Farm; and in 1924 from material kept also at Northfield, Minn. Germination tests were made every month. Table XXIV summarizes the results.

TABLE XXIV

Percentage of Germination of Conidia of *H. sativum*, Form 1, After Having Been Buried One Inch Deep in Soil for Different Periods of Time in 1923, 1924, and 1925

Date of test	University Farm, 1923-24* Outside			Control‡			Date of test	University Farm, 1924-25† Outside			Date of test	Northfield, 1924-25† Outside		
	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.		Ser. A	Ser. B	Av.		Ser. A	Ser. B	Av.
Oct. 4-5	..	..	97	96	98	97	Dec. 4-5	55	33	44	Nov. 14-16	63	65	64
Nov. 7-8	94	96	95	94	97	95.5	Jan. 7-9	91	90	90.5	Dec. 8-9	3	7	5
Dec. 7-8	93	97	95	..	..	..	Feb. 4-5	78	30	54	Jan. 13-15	11	7	9
Jan. 8-10	97	96	96.5	..	..	..	Mar. 4-5	6	12	9	Feb. 10-12	8	11	9.5
Feb. 13-14	98	90	94	95	96	95.5	Apr.	42	54	38	Mar. 15-16	10	12	11
Mar. 9-11	96	95	95.5	97	98	97.5								
Apr. 10-11	99	98	98.5	99	99	99								
May 7-8	90	70	80	99	99	99								
June 12-13	94	96	95	..	..	..								
July 16-18	94	82	88	..	..	..								
Aug. 19-21	83	96	89.5§	96	98	97								
Sept. 15-17	77	52	64.5	98	94	96								

* Put out Oct. 4, 1923.
† Put out Oct. 2 at University Farm and Oct. 5 at Northfield.
‡ Kept in laboratory.
§ Put out Dec. 15, 1923.

Spores exposed outside in October and December, 1923, at University Farm, withstood repeated changes in temperature and humidity, and endured alternate thawing and freezing without appreciable reduction in the percentage of germination until 9 months had elapsed. The results in 1924-25 do not agree with those of the previous year. This might be due to the ecological conditions under which the spores were produced, or it might be due to variation in meteorological conditions. The results indicate the importance of making such tests for more than one year. They also show the marked variation that may be expected in different lots of spores.

That aeration is an important factor in prolonging the life of conidia was demonstrated by the following experiment. Sterilized wheat heads and tubes of potato dextrose were inoculated with *H. sativum*. One series was plugged with cotton only, one was plugged with cotton and capped with tinfoil, and another was sealed with paraffin. These cultures were then put in various places, as indicated in Table XXV. Another lot of spores was produced on sterile wheat heads in a large Erlenmeyer flask and then placed in test tubes. One series was sealed with paraffin, the other, plugged with cotton, was used as a control. A study of the results shows that lack of aeration was more detrimental to spores under the conditions of the experiment than were light and temperature. The writer did not determine whether this deleterious effect was due to lack of oxygen or to production of volatile toxic substances by the organism. The relative humidity might have had some effect, but the results tabulated in Table XXI render this supposition doubtful. It will be remembered that a high percentage of spores germinated at 10° to 12° C. after storage at all relative humidities.

McKinney (47), in 1923, observed that spores of *H. sativum* did not germinate well in large quantities of water. He observed that spores kept in water from April to November germinated well, but toward the end of that period their vitality appeared pretty well exhausted. This is in accord with the writer's results, except that in one lot of spores no apparent decrease in percentage of germination was observed after the spores had been in unsterilized tap water for a year. The results of keeping spores in water are given in Tables XXVI and XXVII.

Spores did not readily lose their viability when kept in distilled water. There were some indications that small amounts of host tissue added to similar quantities of water rapidly reduced the viability of the spores. The reason for this decline is not definitely known, but the water in the containers soon became covered with molds, bacteria, and yeasts. It is quite likely that these organisms inhibited germination.

TABLE XXV

Effect of Aeration on Longevity of Conidia of *H. sativum* When Produced and Stored Under Various Conditions

Place of storage and temperature	Time of exposure, days	Percentage of germination*					Potato dextrose agar
		Wheat heads					
		Tubes plugged with cotton‡	Tubes capped with tinfoil†	Tubes sealed with paraffin†	Tubes plugged with cotton‡	Tubes sealed with paraffin‡	Tubes sealed with paraffin†
5°- 7° C. Dark.........	82	No spores	Few spores	No spores	97.5	94.0	No spores
6°- 9° C. Dark.........	139	...	96.5	...	...	...	...
10°-12° C. Dark.........	82	99.5	68.0	No spores	6.0	98.5	No growths
12°-14° C. Dark.........	129	...	0.0	...	...	...	...
Laboratory Light.........	82	96.5	69.0	0.0	0.0	98.0	No spores
do	139	...	Trace	...	...	...	...
24°-25° C. Dark.........	82	91.0	98.0	0.0	0.0	76.0	Trace
do	139	...	Trace	...	...	...	...
29°-30° C. Dark.........	82	No spores	No spores	No spores	0.0	72.0	No spores
do	139	...	No spores	...	...	...	...
Greenhouse Light.........	82	98.0	93.0	0.0	0.0	...	0.0
Windowsill (south side of building).........	139	...	0.0	...	...	...	...
Windowsill (north side of building).........	139	...	53.0	...	...	...	...

* Percentage based on a count of 200 spores.
† Spores developed after tubes were sealed.
‡ Spores developed before tubes were sealed.

The data in Table XXVI also show that the syracuse dishes to which host tissue was added gave a more reliable index of spore germination than those without the host tissue.

TABLE XXVI

EFFECT ON GERMINATION OF ADDING HOST TISSUE TO WATER IN WHICH SPORES OF *H. sativum*, FORM 12, HAD REMAINED FOR DIFFERENT PERIODS OF TIME WITHOUT GERMINATING

	Percentage of germination and relative vigor							
	Tap water with host tissue				Tap water without host tissue			
Date of test*	Ser. A	Ser. B	Av.	Vigor	Ser. A	Ser. B	Av.	Vigor
1924								
April 9-10	82	72	77.0	Very vigorous	3	2	2.5	Weak
May 9-10	78	86	82.0	do	53	43	48.0	do
June 12-13	85	83	84.0	do	7	17	12.0	do
July 16-18	78	91	84.5	Fairly vigorous	4	45	24.5	Very weak
Sept. 1-2	92	77	84.5	do	35	64	49.5	Weak
Oct. 14-15	91	86	88.5	do	..	..	...	....
Dec. 8-9	85	88	86.5	Vigorous	..	..	...	....
1925								
Jan. 7-8	80	83	81.5	Vigorous	..	..	...	....
Feb. 4-5	92	94	93.0	do	83	77	80.0	Very weak
March 14-15	83	77	80.0	Fair	..	..	...	....

* Experiment started March 10, 1924.

Not only did the spores of Form 12 remain viable in large quantities of water, but they were able to produce typical lesions on wheat and barley after they had been kept under water an entire year.

TABLE XXVII

EFFECT ON VITALITY OF SPORES OF *H. sativum*, FORMS 1 AND 12, RESULTING FROM STORAGE IN WATER CONTAINING HOST TISSUE*

	Percentage of germination											
	Tap water						Tap water with host tissue					
Date of test	Form 1			Form 12			Form 1			Form 12		
	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.
1924												
Oct. 15-16	96	88	92.0	97	98	97.5	53	28	40.5	28	12	20.0
Nov. 10-11	81	83	82.0	96	91	93.5	9	17	13.0	0	0	0.0
Dec. 8-9	70	86	78.0	95	96	95.5	10	6	8.0	4	5	4.5
1925												
Jan. 7-8	79	94	86.5	90	96	93.0	18	22	20.0	7	3	5.0
Feb. 5-6	99	97	98.0	98	97	97.5	12	19	15.5	3	6	4.5
March 15-16	98	98	98.0	98	97	97.5	3	10	6.5	?	T+	T+

* Experiment started September 8, 1924.

The viability of conidia of *H. sativum* when exposed under various conditions has been studied. The data obtained are presented in Table XXVIII. The fungus was cultured on wheat heads, as previously described. The lots exposed in the greenhouse and outdoors were bound in small packets with cheesecloth covering. The sample stored in the greenhouse was attached to a cross-bar by a string. The packet put

outside was suspended beneath the eaves of the greenhouse and thus protected from the rain and direct rays of the sun. Those in the icebox and in the laboratory were put in large open test tubes. The data prove that the conidia of *H. sativum* can retain their viability for months, even years, under diverse conditions if they are kept relatively free from moisture.

XXVIII

Viability of Conidia of *H. sativum*, Form 1, Exposed Under Various Conditions from October, 1922, to December, 1924

Location of spores	Percentage of germination								
	Stored 7 mo.			Stored 17 mo.			Stored 26 mo.		
	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.
Greenhouse (light)	95	95	95.0*	98	96	97.0	86	76	81.0
Under eaves outside (light)	96	92	94.0	27	39	33.0	†	..	...
Icebox (dark)	97	92	94.5	92	91	91.5	54	45	51.0
Laboratory (light)	95	99	97.0	83	97	90.0	81	59	70.0
Check, fresh culture...	..	..	...	99	99	99.0	98	99	98.5

* Percentage for a seven-month period estimated.
† Discontinued.

The writer has previously recorded the fact that spores of *H. sativum* can retain their viability for 23 months (18). Stevens (62) reported that spores were still viable at the end of 14 months. A comparison was made of the viability of conidia of four physiologic forms of *H. sativum* stored under the same and under different conditions for 3 years (Table XXIX). More than 80 per cent of the spores of Form 4 germinated after 3 years on barley heads. There are some indications that the longevity of spores varied somewhat with the physiologic form. It was noted, however, that spores of Form 3 (not indicated in Table XXIX) frequently failed to germinate when kept under conditions similar to those for Form 1. Furthermore, the writer has lost several strains because they failed to grow after stock transfers were made. A successful transfer was made from an old culture obtained from Louise Stakman, after it had been dormant nearly 5 years. It required almost a week for the new colony to become visible to the naked eye. It is apparent from Table XXX that environmental conditions are far more important than differences in physiolgoic forms in determining the longevity of conidia.

TABLE XXIX

PERCENTAGE OF GERMINATION OF CONIDIA OF 4 PHYSIOLOGIC FORMS OF *H. sativum* STORED UNDER THE SAME AND DIFFERENT CONDITIONS FOR THREE YEARS, FROM 1921 TO 1924

Form No.	Environment	Percentage of germination			Vigor
		Ser. A	Ser. B	Av.	
1	On wheat heads and kept in laboratory, at room temperature (dark)	14	17	15.5	Weak, not branched
2	do	12	25	18.5	do
3	do	14	9	11.5	do
4	do	43	40	41.5	Many of germ tubes fairly vigorous
1	On rotted wood in laboratory (dark)	71	81	76.0	Vigorous
2	On potato dextrose agar in petri dishes in laboratory (dark)	0	0	0.0	
4	On barley heads in laboratory (dark)	85	81	83.0	Fairly vigorous
1	On wheat straw in a flask (light)	0	0	0.0	

OVERWINTERING OF CONIDIA

Previous studies (18) have shown that the mycelium of *H. sativum* overwinters in the seed and on plant remains in the field. Spores produced on agar and then put outdoors did not germinate the next spring. Henry (37), however, found that a rather high percentage of conidia of *H. sativum* overwintered. It has already been mentioned (Table XXVI) that conidia of *H. sativum* put outdoors in the fall remain viable until the fall of the following year. In the present work, overwintering studies were made for 4 years. Conidia were put outdoors in the fall, and germination tests were made the following spring. The results for the last 3 years are summarized in Table XXX. The results show clearly that spores of *H. sativum* overwinter under varying conditions in the vicinity of St. Paul. The mortality of the conidia varied from one year to another, altho placed in the same locality each year. In the same year there was a great variation in the percentage of spores that remained viable after being buried at different depths in the soil.

In 1923, 2.5 per cent of the spores germinated when buried from 6 to 8 centimeters in the soil. In 1924, 81.5 per cent germinated; and in 1925, 17 per cent. Similar fluctuations were obtained also for spores stored on the surface and buried just below the ground-line.

The results of the writer's studies on the vitality and germination of spores indicate clearly that the pathogene is well fortified against adversity. The spores can withstand low as well as high temperatures if the relative humidity is favorable. Furthermore, they can withstand long periods of alternate freezing and thawing. They can even remain in water for a year or longer without germinating. When host tissue is added to a small quantity of water, however, the spores germinate promptly. This suggests the possibility that in nature the spores germinate readily only in contact with host tissues.

TABLE XXX

Environment	Percentage of germination*								
	1922-23			1923-24			1923-25		
	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.
Kept under eaves 12 feet from ground	80	90	85	..	..	...	92	90	91
Covered lightly with white sand next to green-house	20	15	17.5	99	99	99.0	30	48	39
On surface	5	T	2.5+	55	81	68.0	15	19	17
Buried 6-8 cm.	2	T	1.0+	82	81	81.5	90	88	89
Buried 12-16 cm.	75	95	85.0	87	90	88.5	0	0	0
Buried 25-30 cm.	..	..	...	91	96	93.5	0	0	0

* Percentage for 1922-23 estimated.

EFFECT OF ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF THE DISEASE

SEASONAL VARIATION

Field observations and numerous isolations for the last 5 years indicate that the Helminthosporium disease of cereals is present every year in practically every field of wheat and barley in Minnesota, but it is much more destructive in some years than in others (Tables XXXI and XXXII).

In 1919 a severe seedling blight of wheat developed in the spring-wheat region. In 1921 a severe epidemic of root-rot and basal stem-rot developed on a large number of barley varieties at University Farm, but in 1922 the infection on the same varieties was rather weak. In 1924 barley was infected only slightly, while wheat was severely damaged by root- and basal stem-rot. In 1921 and 1923 considerable seed infection occurred in wheat, but in 1924 the seeds were either clean or only slightly infected at harvest time. Data in Table XXXII show that there was the same relative variation in the amount of foliage infection of barley. Thus it is apparent that certain ecological factors influence profoundly the severity of the disease. A knowledge of these factors, obviously, is necessary for an effective control.

TIME OF INFECTION

Infection can occur under conditions which do not favor the development of the disease. Dosdall (24) found that germ tubes of *H. sativum* penetrated the tissue of both coleoptile and leaf at temperatures ranging from 12° to 34° C. However, the fungus may remain quiescent in host tissues for weeks under unfavorable temperature conditions.

TABLE XXXI

VARIATIONS IN REACTION OF WHEAT VARIETIES TO *H. sativum* GROWN ON INOCULATED SOIL FOR 4 YEARS AT UNIVERSITY FARM

Species and varieties	C.I. No.	Root- and basal stem-rot				Seed blight			
		1921	1922	1923	1924	1921	1922	1923	1924
Triticum vulgare									
Dicklow	3663	M—	M—	M—	M	L+	T	T	o
Glyndon	2870	M—	L—	M—	L+	L	o	T—	o
Huron	3315	M	L	L+	L	L	o	o	o
Marquis	3641	M—	L	L—	M	L—	T	T	o
Haynes Bluestem	2874	M—	T+	T	L+	L+	o	T	T—
Bobs	4991	M	M—	M	M—	M+	T	M—	o
Kota	5878	L	T+	L+	L	L	o	T—	o
Preston	3328	M—	L—	L+	L	L	o	T	o
Prelude	4323	L+	L	M+	..	L+	T—	o	..
Sonora	3036	M	T+	M	L+	H	T+	M	T—
Kitchner	4800	M	L—	L+	L+	L+	o	T—	T—
T. compactum									
Little Club	4066	M	L	L	L+	M—	No seed	T—	T
T. dicoccum									
Khapli	4013	M	L	H—	H	T	o	T+	T—
T. turgidum									
Alaska	5988	M+	T+	M—	M	M	T	o	T—
T. durum									
Acme	5284	M	L+	M	M+	H	L	L+	T
Iumillo selection	....	M—	L—	H—	M	M	T	M—	T—
Kahla	5529	M+	L—	L+	H	H	T	T+	L—
Monad	3320	M+	L+	M	H—	H	L+	L—	T
Pentad	3322	H—	M—	M	M+	H+	M—	M	T
Peliss	1584	M—	T+	M—	M	H—	L—	M+	T—

* T=trace.   L=light.   M=moderate.   H=heavy.

TABLE XXXII

VARIATIONS IN REACTION OF BARLEY VARIETIES TO *H. sativum* GROWN ON HELMINTHOSPORIUM SICK SOIL FOR 4 YEARS

Species and varieties of barley	Root- and basal stem-rot				Spot blotch			
	1921	1922	1923	1924	1921	1922	1923	1924
Hordeum vulgare								
Arequipa (selection)	H+	L—	M+	L—	H+	T+	M+	L—
Bay Brewing do	H+	L—	M	L—	H+	T—	M+	L—
Manchuria Minn. 184	L+	T+	L+	T+	L+	T	L	T
Minsturdi Minn. 439	M—	L—	L	T+	H	T+	L+	L—
Mariout (selection)	M	H	M	L—	H	M—	H+	L+
Nepal do	M+	L+	M	L—	H	L	M—	T+
N. S. Wales do	M	..	H—	L	H—	..	H	L+
Trebi do	L+	L	M+	L—	M—	T+	M	T+
Lion do	M	M+	H—	L	H+	L+	M+	L—
H. distichon								
Chevalier Minn. 230	M	L—	L	T+	H—	T+	L—	T+
Hanna (selection)	M+	T+	M—	T+	H+	T+	L+	T+
Hannchen do	M+	T+	M	T+	H+	T	L+	T+
Svanhals do	M	L—	L—	T	M+	T+	L	T
Svansota Minn. 440	M—	T+	L	T	M	T	L+	T

* T=trace.   L=light.   M=moderate.   H=heavy.

Unfavorable external and internal conditions may prevent the pathogene from becoming destructive. At St. Paul, primary lesions of *H. sativum* have been observed on the underground parts of wheat and barley sown at intervals from early April to the middle of October. Under favorable conditions secondary infections may occur repeatedly during the life of the host. Thus, the appearance and the severity of the disease depend on an environmental complex. The temperature must be favorable and a certain amount of moisture is necessary; the spores must germinate, or resting mycelium must resume its growth. Under certain conditions the fungus may grow so slowly and the host so rapidly that it may grow away from the pathogene. This, in part, accounts for some of the variations in infection observed from season to season.

INFLUENCE OF SOIL MOISTURE

The influence of soil moisture on the development of the disease on cereals at various stages of growth is imperfectly known. Dosdall (24) concluded that root-rot and basal foot-rot were more severe in extremely dry or wet soil than in soil containing an optimum amount of moisture for the growth of the plant. McKinney (47) concluded that at soil temperatures of $24°$ C. or higher, the disease developed better in moist soil, and that at temperatures below $24°$ C., low soil moisture content favored the disease.

In order to obtain additional information on the influence of soil moisture, 2 varieties of wheat and 4 varieties of barley were sown on peat with five different drainage levels. The wheat varieties used were Monad (C. I. 3320) and Pentad (C. I. 3322); the barley, Minsturdi (Minn. No. 439), Svansota (Minn. No. 440), Manchuria (Minn. No. 184), and Lion selection. The water levels were approximately 1, 2, 3, 4, and 4.5 feet below the surface. Duplicate rows 33 feet long of each of the varieties were sown on each water level in 1923 and 1924. There was a space of 33 feet between each drainage level. Notes were taken on the degree of *Helminthosporium* infection and yield. Soil temperature readings and soil moisture determinations were made at irregular intervals.

A severe epidemic of the disease developed in both years. In 1923 there was considerable seedling blight in all plots. Root-rot and basal stem-rot were most serious for all varieties on the 4- and 4.5-foot water levels. Practically all the Monad and Pentad wheat plants were killed on the 4.5-foot level, and from 50 to 70 per cent of the barley plants. In 1924 the epidemic developed much later in the season. Monad and Pentad wheats were severely damaged on all drainage levels except the 1-foot level. The data on the number of plants killed by root- rot and basal stem-rot at two different dates are presented in Table XXXIII.

The plants in Series A which were inoculated with *H. sativum* succumbed first to the attack of the organism. In platings from diseased basal stems of uninoculated plants, 87 per cent of the colonies were *H. sativum,* indicating that this organism was the principal pathogene. Practically all the plants on the 2- to 4.5-foot drainage levels were killed after heading. Those surviving were small, diseased, and weak. At least four-fifths of the plants on the 1-foot water level developed normally and produced plump grains, altho sown late in the spring.

TABLE XXXIII

COMPARATIVE NUMBERS OF WHEAT PLANTS KILLED IN 1924 BY ROOT- AND BASAL STEM-ROT ORGANISMS WHEN GROWN ON PEAT SOIL WITH FIVE DIFFERENT DRAINAGE LEVELS

Drainage levels, feet	Variety	Percentage of culms dead*				Remarks
		Series A†		Series B		
		Aug. 28	Sept. 9	Aug. 28	Sept. 9-24	
1	Monad	14	15	13	17	Heads large, plants upright
1	Pentad	12	12	10	20	do
2	Monad	20	96	12	85	Weak plants lodged
2	Pentad	23	95	11	69	do
3	Monad	29	98	18	89	do
3	Pentad	23	99	14	74	do
4	Monad	22	93	10	86	do
4	Pentad	31	98	9	86	do
4.5	Monad	14	97	11	59	Most of living plants late, small
4.5	Pentad	15	95	11	58	do

* Percentage on August 28 based on a count of 400 culms; on September 9, on 200 culms for each series.

† Series A, soil inoculated with *H. sativum;* series B, control.

The relative amount of water in the six different drainage levels at from 1 to 2 inches below the surface was approximately the same for all plots except the 1-foot level (Table XXXIV) which contained an average of 100 per cent more moisture than the others. The percentage of Monad and Pentad wheat plants killed is correlated negatively with the amount of moisture present in the soil. It is interesting to note that two varieties of durum wheat could grow apparently normally in peat soil saturated with moisture.

TABLE XXXIV

RELATIVE AMOUNTS OF WATER IN PEAT SOIL WITH FIVE DIFFERENT DRAINAGE LEVELS, 1924

Drainage level, feet	Percentage of moisture at a depth of from 1 to 2 in.				
	July 8	July 17	Aug. 28	Sept. 9	Average
1	303	300	327	405	333.7
2	199	196	238	126	189.7
3	184	181	217	123	176.2
4	195	183	208	233	204.7
4.5	191	184	206	176	189.2

Temperature readings of peat soil with five different drainage levels were taken at different times and on different dates, as shown in Table XXXV. Readings were made at identical places in the six different series. Three separate readings were made on every plot for each test. The results show a uniformity of temperature on all levels at the time the readings were made. The data also show that the temperature of peat soil may fluctuate several degrees during a few hours.

TABLE XXXV

AVERAGE COMPARATIVE TEMPERATURES* OF PEAT SOIL ON FIVE DIFFERENT DRAINAGE LEVELS
IN 1924

Date of readings	Depths of readings, cm.	Temperature (° C.) at different water levels				
		1 foot	2 feet	3 feet	4 feet	4.5 feet
June 25	6-7	23.8	24.5	24.3	24.3	24.8
June 25	2-3	30.8	30.6	30.0	29.5	31.6
Sept. 2	6-7	20.3	20.1	20.1	20.5	20.3
Sept. 2	2-3	23.8	23.1	23.6	22.8	23.8
Sept. 9						
10-11 a.m.	6-7	13.1	13.0	13.1	13.1	13.1
3-4 p.m	6-7	18.8	19.0	18.8	19.1	20.0
Mean average	..	21.7	21.7	21.6	21.5	22.2

* Average of three readings in different locations.

The rate of growth of fungi on culture media was used to determine the possible constancy in temperature at different water levels over a period of 13 days. Large test tubes, 25 cm. by 2.5 cm., containing 20 cc. of potato dextrose, were slanted so as to give a lateral surface for growth of at least 15 cm. Twelve of these tubes were inoculated in the center of each slant. Two of the tubes were buried on each water level in different places from 6 to 8 cm. beneath the surface of the soil. After 13 days they were dug up and the radial spread of the colonies was measured. Table XXXVI contains the data. The greatest difference in radial spread was 0.05 cm. in tubes which were buried in the 3- and 4-foot water levels. The differences in development of cultures at high and low drainage level is only 0.01 cm. This surely is not significant.

TABLE XXXVI

COMPARATIVE RATE OF SURFACE GROWTH OF *H. sativum* ON POTATO DEXTROSE AGAR WHEN
BURIED 6-8 CM. IN PEAT SOIL WITH FIVE DIFFERENT DRAINAGE LEVELS

Depth of drainage, feet	Diameter of colonies, cm.*			Av. growth of colonies per day, cm.
	Ser. A	Ser. B	Av.	
1	3.5	3.3	3.40	0.26
2	3.2	3.2	3.20	0.24
3	3.2	3.3	3.25	0.25
4	4.2	3.8	4.00	0.30
4.5	3.6	3.5	3.55	0.27

* Period of 13 days—June 25 to July 8, 1924.

The data in Tables XXXV and XXXVI seem to prove that the slight variation in temperature at the six different water levels can not account for the differences in the killing of the two varieties of wheat grown there. The variation in the percentage of killing, therefore, would probably have to be explained primarily on the basis of soil temperature. The basal stems of diseased plants were usually discolored. The roots adjoining them were often badly diseased or rotted. It is possible that the water supply of the diseased plants was materially reduced. The roots and stems of the plants on the 1-foot water level usually were not so distinctly discolored as those in the other plots. It seems safe to conclude that high soil moisture either inhibits the growth of *H. sativum* or renders the plants less susceptible. Perhaps the proximity of the moisture enables the plants on the 1-foot water level to obtain sufficient moisture during the critical period, which insures normal filling of the kernels. In 1924 the wheat varieties were a total failure on all except the 1-foot level. In general the varieties of barley did equally well on the 2-, 3-, and 4-foot drainage levels in 1923. In 1924 the barley varieties yielded fairly well, also, on the 4.5-foot level. These results indicate that wheat and barley respond differently to soil moisture. Wheat with basal stem-rot developed best on plots with relatively shallow surface drainage, and barley on the plots with intermediate surface drainage.

In 1923 and 1924 Minsturdi barley produced the highest mean yield in all plots. The yields undoubtedly would have been even higher except for the fact that in both years from 5 to 10 per cent of the plants were infected with *H. gramineum*. No attempts were made to control the disease. Manchuria, Minn. No. 184, was very susceptible to freckles, a physiologic disease which caused considerable damage at times to varieties of barley grown on peat, especially Manchuria. The results indicate that the lowering of soil moisture to a point where metabolism of the wheat or barley plant becomes abnormal, predisposes them to attack by *Helminthosporium*. This statement is supported by laboratory tests and field observations. *Helminthosporium* disease is destructive on peat soil because the organism will grow and fructify on the organic matter in peat, and because insufficient or unbalanced nutrition predisposes the host plants. Heavy dews favor foliage and spike infection. A paucity of available soil moisture inhibits the growth of the diseased plants. It is apparent from these experiments and observations that foliage infection, root- and basal stem- rot caused by *Helminthosporium* and other organisms are limiting factors in cereal production on peat soil.

EFFECT OF TIME OF PLANTING ON DEVELOPMENT OF
H. SATIVUM

In order to obtain data on the influence of temperature on the *Helminthosporium* disease and on the relation of the degree of susceptibility of the host plants in various stages of development, several sowings of wheat and barley were made at various intervals. Each sowing consisted of 3 seven-foot rows of each variety. In 1924 Marquis, C. I. 3641, and Monad, C. I. 3320, wheats; and Manchuria Minn. No. 184, and Lion selection barleys were used. In 1923 only the barley varieties were sown. In 1924 notes were taken at intervals as indicated in Tables XXXVII and XL. The soil was inoculated on the date of sowing by applying inoculum grown on sterilized wheat kernels.

Table XXXVII indicates that environment influences the development of seedling blight and basal stem-rot to a much greater extent than does the stage of host development. Thus, early-sown Lion and Manchurchia barleys were infected only slightly, but the same varieties sown on June 4 became heavily infected with root-rot. Fifty per cent of the Manchuria plants sown on July 9 were killed before they reached the boot stage. For several weeks the early-sown wheat plants remained relatively free from root-rot. However, as the season advanced and these plants began to mature, they developed a severe root- and basal stem-rot. Field observations indicate that the barley and wheat varieties pass through a stage in which they are somewhat resistant to root- and basal-stem rot. This period of apparent resistance extends from the time of development of adventitious or permanent roots, to the heading stage. From the milk stage to maturity, root-rot, and (especially) basal stem-rot, developed very rapidly.

The degree of infection depends to a marked extent upon the host and the environmental conditions. Henry (37) is of the opinion that adventitious roots are more resistant than seminal roots to many root-rot pathogenes. This resistance is not absolute, because the killing of plants in all stages of development by *H. sativum* is not uncommon.

In the winter of 1923 more than a thousand pots of wheat from 2 to 4 weeks old were inoculated with cultures of *H. sativum* in the greenhouse. In every case the roots and basal portion of the stem became diseased. Some of the plants were stunted and many died throughout the course of the experiment.

Results given in Table XXXVIII show that the greatest percentage of plants killed after heading occurred in the plots sown early in the season. Only a few of the plants sown after the middle of June succumbed. The mortality of the plants was greatest during the latter part of July and the first two weeks in August. The plants continued to die prematurely up to the hard dough stage. In every case Monad was injured to a much greater extent than Marquis.

TABLE XXXVII

Degree of Foliage Infection on Barley and Root- and Basal Stem Rot Infection for Marquis and Monad Wheat and Manchuria and Lion Barley Grown on Artificially Inoculated Soil*

Host	Date sown	June 6 Leaf spot	June 6 Root-rot	June 6 Stage, in.	July 1 Leaf spot	July 1 Root-rot	July 1 Stage, in.	July 21 Leaf spot	July 21 Root-rot	July 21 Stage, in.	Aug. 25 Leaf spot	Aug. 25 Root-rot	Aug. 25 Stage, in.	Sept 2 Root-rot	Remarks
Manchuria	Apr. 30	T−	T−	14	T−	T−	H	T	T+	HD					
Lion	do	T	T−	12	T+	T−	H	L	T−	HD					
Marquis	do	....	T−	12	....	L−	B	....	L−	SD	....	L+	R	....	
Monad	do	....	T	10	....	L	J	....	L	SD	....	H	R	....	
Manchuria	May 12	T−	L−	8	T−	T−	Hg	T	T+	SD					
Lion	do	T−	L−	8	T	T+	Hg	L	T+	SD					
Marquis	do	....	T+	6	....	T+	J	....	L−	M	....	M−	R	....	
Monad	do	....	M−	6	....	L−	16	....	L−	M	....	H	R	....	
Manchuria	May 15	T−	T	10	T	T	B	T	T	SD					
Lion	do	T−	T	8	T+	L−	Hg	L−	T+	SD					
Marquis	do	....	T	8	....	L+	J	....	L−	M	....	M	R	....	
Monad	do	....	L−	7	....	L−	J	....	L−	M	....	H	R	....	
Manchuria	May 21	T−	T	7	T−	T−	J	T−	L−	SD					
Lion	do	T	T−	5	T+	T+	B	T−	L	SD					
Marquis	do	....	L+	6	....	L	J	....	L	FI	....	M+	R	....	
Monad	do	....	L+	6	....	L	J	....	L+	FI	....	H	R	....	
Manchuria	May 28	0	T−	5	T−	L−	J	T	L−	M					
Lion	do	0	T	4	T+	L	M	T+	L−	M					
Marquis	do	....	T	4	....	L	M	....	L	FI	....	M	R	....	
Monad	do	....	T	4	....	L+	Hg	....	L+	Hg	....	H	HD	....	

TABLE XXXVII *Continued*

| | | Degree of infection and stage of host* | | | | | | | | | | | | | |
| Host | Date sown | June 6 | | | July 1 | | | July 21 | | | Aug. 25 | | | Sept 2 | Remarks |
		Leaf spot	Root-rot	Stage, in.	Leaf spot	Root-rot	Stage, in.	Leaf spot	Root-rot	Stage, in.	Leaf spot	Root-rot	Stage, in.	Root-rot	
Manchuria	June 4	0	T–	3	T–	T–	10	T–	L	Fl	....	H–	....	....	....
Lion	do	0	T–	2	T–	T+	7	T	M–	Hg	....	H–	....	....	....
Marquis	do	....	T–	3	....	L+	6	....	M–	B	....	H	HD	....	....
Monad	do	....	T–	3	....	L+	7	....	M	D	....	H	HD	....	....
Manchuria	June 12	....	....	....	T–	T–	5	T–	L+	B	L	H	HD	....	....
Lion	do	....	....	....	T–	T–	5	T	L+	B	M–	H	HD	....	....
Marquis	do	....	....	....	....	T+	4	....	M–	J	....	M–	SD	....	....
Monad	do	....	....	....	....	T–	4	....	M	J	....	M	M	....	....
Manchuria	June 18	....	....	....	0	L–	3	T	L	J	L+	H–	HD	....	....
Lion	do	....	....	....	T	T	3	T	L+	J	M	H	HD	....	....
Marquis	do	....	....	....	....	L	3	....	M–	8	....	H–	M	H–	Poor stand
Monad	do	....	....	....	....	L+	3	....	M+	6	....	H	Fl	H–	
Manchuria	June 25	....	....	....	0	0	1	T–	M–	6	L–	H	M	H–	
Lion	do	....	....	....	0	0	1	T–	M–	4	M	H	SD	H–	
Marquis	do	....	a	....	....	0	1	....	M+	4	....	H	T	H	75% of plants killed mostly
Monad	do	....	....	....	....	0	1	....	M+	4	....	H	Hg	H	in seedling and jointing stage
Manchuria	July 2	....	....	....	....	....	....	T–	M–	6	T+	M–	Hg	H	
Lion	do	....	....	....	....	....	....	T–	M–	4	M	M	Fl	H	25% killed
Marquis	do	....	....	....	....	....	....	....	M+	4	....	H+	B	H	
Monad	..	....	....	....	....	....	....	....	M+	3	....	H+	J	H	75% killed
Manchuria	July 9	....	....	....	....	....	....	o	T+	3	T	L+	J	L	50% killed
Lion	do	....	....	....	....	....	....	o	L+	3	T+	L+	Fl	L+	20% killed
Marquis	do	....	....	....	....	....	....	....	T+	2	....	H	J	L+	50% killed
Monad	do	....	....	....	....	....	....	....	T+	2	....	H	8	L	10% killed

TABLE XXXVII—*Concluded*

Host	Date sown	June 6			July 1			July 21			Aug. 25			Sept 2	Remarks
		Leaf spot	Root-rot	Stage, in.	Leaf spot	Root-rot	Stage, in.	Leaf spot	Root-rot	Stage, in.	Leaf spot	Root-rot	Stage, in.	Root-rot	
Manchuria	July 21	....	....	....	....	....	....	....	....	....	T	L+	8	L	Good stand
Lion	do	....	....	....	....	....	....	....	....	....	L+	L+	8	L	do
Marquis	do	....	....	....	....	....	....	....	....	....	....	H	6	L	20% killed†
Monad	do	....	....	....	....	....	....	....	....	....	....	H	6	L	10% killed†
Manchuria	July 28	....	....	....	....	....	....	....	....	....	T	L—	6	T+	
Lion	do	....	....	....	....	....	....	....	....	....	T	L—	6	T+	Very good stand
Marquis	do	....	....	....	....	....	....	....	....	....	....	M—	4	T+	
Monad	do	....	....	....	....	....	....	....	....	....	....	M—	4	T+	
Manchuria	Aug. 6	....	....	....	....	....	....	....	....	....	T—	L—	6	T—	
Lion	do	....	....	....	....	....	....	....	....	....	T—	L—	6	T—	Vigorous growth and excellent stand
Marquis	do	....	....	....	....	....	....	....	....	....	....	T	6	T—	
Monad	do	....	....	....	....	....	....	....	....	....	....	T	6	T—	
Manchuria	Aug. 15	....	....	....	....	....	....	....	....	....	o	T—	4	T—	
Lion	do	....	....	....	....	....	....	....	....	....	o	T—	4	T—	do
Marquis	do	....	....	....	....	....	....	....	....	....	....	T+	4	T—	
Monad	do	....	....	....	....	....	....	....	....	....	....	T+	4	T—	

* Symbols expressing stages of development:

B=Boot   Hg=Heading   Fl=Flower   SD=Soft dough   R=Ripe

J=Jointing   H=Headed   M=Milk   HD=Hard dough

† Plants recovering.

A study of Table XXXVII shows that very little foliage infection occurred during the summer of 1924. The low foliage infection is correlated with a low percentage of seed infection of wheat (Tables XXXI, XXXII, and XLIX). In 1921, when barley leaves were heavily infected, there was also a severe seed blighting of wheat. Heavy foliage infection in 1921 seemed to be correlated with a combined high mean temperature and high precipitation for the month of July (Table XXXIX). The small grains were harvested during this month. In 1923 a moderate foliage infection developed on barley varieties. In that year July was hot, but the amount of precipitation was not high. The mean temperature during July in 1922 and 1924 was low. In these two years the foliage infection also was low. The data given in Table XXXIX seem to indicate that a high temperature combined with a moderate to high rainfall favors abundant foliage and seed infection.

TABLE XXXVIII

Results of Planting Wheat on Different Dates in Relation to the Number of Culms Killed by Root- and Basal Stem-Rot on Soil Artificially Inoculated with *Helminthosporium,* 1924

Date of planting	Variety	Percentage of culms dead on different dates*			Stage of development on different dates		
		Aug. 13	Sept. 2	Sept. 11	Aug. 13	Sept. 2	Sept. 11
April 30	Marquis	14	....	....	Ripe	....	....
do	Monad	92	....	....	do	....	....
May 12	Marquis	15	....	....	do	....	....
do	Monad	84	....	....	do	....	....
May 15	Marquis	14	....	....	do	....	....
do	Monad	90	....	....	do	....	....
May 21	Marquis	8	....	....	Hard dough	....	....
do	Monad	26	....	....	do	....	....
May 28	Marquis	11	....	....	Soft dough	....	....
do	Monad	21	53	....	do	Ripe	....
June 4	Marquis	2	8	....	do	do	....
do	Monad	8	35	....	Milk	Hard dough	....
June 12	Marquis	1	3	3	do	do	Ripe
do	Monad	3	12	20	Flower	Soft dough	Hard dough
June 18	Marquis	0	3	3	do	do	do
do	Monad	0	4	6	Headed	Milk	Soft dough
June 25	Marquis	....	0	T—	Heading	do	do
do	Monad	....	0	T	Boot	Flower	Milk
July 2	Marquis	....	0	0	....	do	do
do	Monad	....	....	0	....	Boot	Heading

* Plants that have headed out, seedling blight not considered.

TABLE XXXIX

PREVAILING WEATHER CONDITIONS AT ST. PAUL FOR FOUR MONTHS, 1919-24*

Year		Mean temperature				Total precipitation			
		May	June	July	August	May	June	July	August
		Deg.	Deg.	Deg.	Deg.	In.	In.	In.	In.
1919	....	57.5	69.1	72.7	68.2	2.17	4.75	6.15	1.41
1920	....	59.0	68.0	70.2	69.2	2.34	9.64	1.35	0.96
1921	....	59.8	73.5	76.7	70.0	4.10	3.19	4.27	1.05
1922	....	62.5	68.3	68.8	72.0	2.86	6.76	1.73	1.55
1923	....	58.6	70.0	75.2	66.9	3.05	4.95	2.90	1.90
1924	....	49.8	63.6	69.0	67.0	1.47	7.24	1.73	6.51
Average	....	57.8	68.7	72.1	68.8	2.66	6.08	3.02	2.23

* Data obtained from U. S. Dept. Agr. Weather Bureau.

Tables XXXVII and XL both indicate that variation in foliage infection was not correlated to any great extent with stages of development. In general, a higher degree of infection occurs on more advanced plants, e.g., those in the soft or hard dough stage, than on young seedlings. This probably is because older plants were exposed to inoculation for a longer time than the seedlings. There is some evidence that leaves on small barley seedlings are less susceptible than those on older plants (18 and 60). Seedling blight is not nearly so detrimental to grain culture as the basal stem-rot which occurs later in the season. Unless the blight results in the killing of plants in definite patches, the plants that survive the seedling blight will often tiller to a much greater extent than normally and thus counteract the loss in stand resulting from seedling blight. It is, however, of great importance to remember that when seedling blight is severe, necrosis of the secondary culms frequently results before they emerge from the ground. Further, this in turn is often followed by considerable rot of permanent roots and of the bases of culms. The fact that many seedlings are also stunted early in the season and often apparently recover must not be overlooked. These plants can by no means yield as much or produce as good grain as plants which have grown normally.

TABLE XL

Variety	Date planted		Stage of development*	Degree of root and basal stem infection	Degree of leaf infection
Manchuria	Apr.	27	Ripe	M	L
Lion	"	27	do	H—	H
Manchuria	May	1	do	M—	L+
Lion	"	1	do	H—	H—
Manchuria	"	5	Practically ripe	L+	L—
Lion	"	5	do	M+	M+
Manchuria	"	9	do	L	L
Lion	"	9	do	M	H
Manchuria	"	12	Hard dough	L+	L—
Lion	"	12	do	M	H—
Manchuria	"	16	Headed	L	L—
Lion	"	16	do	M—	H—
Manchuria	"	25	Heading	L	L
Lion	"	25	Just headed	L+	M
Manchuria	June	1	Boot stage	L	M
Lion	"	1	do	L+	M+
Manchuria	"	9	Seedlings	L—	M—
Lion	"	9	Boot stage	L+	M+
Manchuria	"	16	Seedlings	T+	M—
Lion	"	16	do	L—	M+

* Notes were taken July 19, 1923.

EFFECT OF TIME OF PLANTING ON DEVELOPMENT OF
H. GRAMINEUM

Reference to Table XLI and Figure 2 will show that early sowing in the spring increases the amount of barley stripe infection. The data also show that no infection occurs during the hottest part of the summer, but the disease develops again in the latter part of the summer and early fall. Thus the two diseases caused by *H. sativum* and *H. gramineum* are influenced differently by temperature. Johnson (39) has recently found that low soil temperatures (10° to 12° C.) favor infection of barley by *H. gramineum* Rab. under controlled experimental conditions. Very little stripe occurred at soil temperatures higher than 20° C. McKinney (47), Henry (37), and others have concluded that high temperatures are conducive to seedling infection caused by *H. sativum*. Dosdall (24) demonstrated that rather high temperatures were most favorable for the growth of the organism, for spore germination, and for the development of the disease.

TABLE XLI

EFFECT OF DATE OF SOWING ON DEVELOPMENT OF BARLEY STRIPE IN 1923 AND 1924

Date of sowing		Percentage of stripe*	
1923	1924	1923	1924
Apr. 27	Apr. 30	14.0	20.2
May 1	May 12	13.5	15.8
" 4	" 15	17.0	17.2
" 9	" 21	7.5	12.8
" 12	" 28	9.5	4.2
" 17	June 4	2.0	4.0
" 21	" 12	0.	0.6
" 25	" 18	0.	0.
" 31	" 25	0.	0.
. . . .	July 2	. . .	0.
. . . .	" 9	. . .	0.
. . . .	" 21	. . .	0.
. . . .	" 28	. . .	0.6
. . . .	Aug. 6	. . .	1.2
. . . .	" 14	. . .	4.2
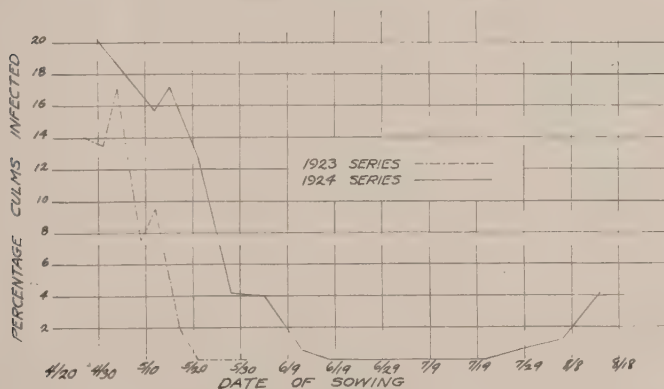
Fig. 2. Effect of Date of Sowing on Development of Barley Stripe in 1923 and 1924

PREDISPOSITION OF THE HOST

The influence of moisture and temperature on the development of the pathogene and the disease has been discussed in some detail. It may be that the influence of these factors, especially temperature, is more important on the host than on the organism. High temperatures are favorable to the fungus but unfavorable to wheat and barley. Dickson's work (22) and that of Eckerson and Dickson (27) and others tend to indicate that the temperatures which predispose the host are more important than those which influence the development of the pathogene. It has been shown that *Gibberella saubinetii* blights corn most severely at low temperatures and wheat at relatively high temperatures.

Predisposition plays an extremely important part in the degree of infection of *H. sativum*. Even the most resistant varieties known may be severely attacked under conditions unfavorable for the host but favorable for the pathogene. Thus, during the summer of 1923, Manchuria, Minn. No. 184, a resistant variety of barley (33), was so severely attacked by *H. sativum* on peat soil that many plants were killed in the seedling stage and plants continued to die throughout the season. Two other varieties of barley and 3 of wheat were similarly attacked (Table XLII).

TABLE XLII

EFFECT OF SOIL TYPE ON DEVELOPMENT OF FOOT-ROT AND ROOT-ROT OF WHEAT AND BARLEY IN 1923 AND 1924

	Degree of infection			
Cereal and variety	Hempstead l.am, University Farm		Peat soil,* Purgo swamp	
	1923	1924	1923	1924
Wheat				
Monad, C. I. 3320............	M	H—	H+	H+
Marquis, C. I. 3641.........	L—	M	H	H—
Pentad, C. I. 3322..........	M	M+	H+	H+
Barley				
Manchuria, Minn. No. 184...	L	T	H+	M—
Lion selection	H	L—	H+	M—
Svansota, Minn. No. 440....	L	T	M+	L+

* On 4.5-foot drainage level.

The injury was chiefly due to leaf infection and to basal stem-rot. The roots, in general, were not severely attacked. In these tests Manchuria exhibited no real resistance, which emphasizes the fact that resistance to *H. sativum* is only relative and not absolute.

Since the organism is usually present in the soil or on the seed, and as spores are blown long distances by the wind, nearly all wheat and barley plants eventually become infected with the organism. When a resistant host is weakened by unfavorable conditions, it usually becomes severely infected. The reaction of the soil determines to a great degree the extent of infection. The disease was always worse in alkali spots than on land immediately adjoining them. This was undoubtedly due to the fact that the host had become weakened rather than to the effect of soil reaction on the pathogene.

There are great variations in the severity of *Helminthosporium* root-rot and basal stem-rot in adjoining fields. Some fields are practically free from the disease, while others are severely infected. There are all gradations between these extremes. Altho the variety of grain grown may be the same and the topography of the fields identical, the disease is nearly always most destructive in poorly tilled fields.

Field observations and experiments indicate that the disease is most destructive on the underground parts of the plants in peat soils and sandy soil. Data in Table XLIX show that the disease caused more damage to roots of plants on sandy soil than to those on heavier soils. This was the case in 1923. But in 1924, there was as much root infection in one type of soil as in the other. An early drying-out of the sandy soil in 1924 brought about a premature death of the plants as well as a cessation of the activity of the fungus.

The apparently great susceptibility of wheat and barley to basal stem-rot on sandy soil may be explained partly by the effect of temperatures on the host, rather than on the fungus. Soil temperature readings were made on sandy soil at Coon Creek, July 12, 1924, between the rows of cereals, one foot apart and at a depth of one inch. The temperature was high throughout, ranging from 44° to 48°, and altogether too high for the normal functioning of the plants.

From the above data and discussion, it is evident that the extent to which wheat and barley plants are affected by *Helminthosporium* depends on such environmental factors as air temperature, soil moisture, aeration, and precipitation. The host reaction depends also upon the type of soil, the amount of plant food available, the tilth of the soil, and the soil temperature. The degree of infection was influenced profoundly by the amount of inoculum, the physiologic form of *H. sativum* concerned, and the genetic constitution of the host plants involved.

EFFECT OF SEED TREATMENT ON THE PATHOGENE AND THE HOST

EFFECT OF SEED TREATMENT ON GERMINATION

There are rather conflicting reports on the effect of various fungicides upon seed germination (3, 45, 55, and 66). In order to obtain additional information, the following experiment was carried out: 3 varieties of wheat, Marquis, Monad, and Pentad; and 4 varieties of barley, Arequipa, Lion, Manchuria, and Svansota, were treated with six chemical dusts and ten liquid fungicides. Germination tests were made in the greenhouse on flats 3 x 4 feet, containing a mixture of sand and soil infected with pathogenic fungi obtained from an experimental plot. Each treatment was tried in duplicate rows, 100 seeds being sown to each row. These comparative germination tests are summarized in Tables XLIII and XLIV.

A study of the data will reveal in many cases appreciable increase in germination, especially for wheat treated with the liquid mercuric compounds. The writer realizes that these treatments should have been repeated several times and statistical methods applied in order to be conclusive. The differences between the control and the treatment in many cases were consistent and warrant some discussion. Chlorophol, Semesan, Uspulun, silver nitrate, and Tillantin greatly increased the percentage of germination of wheat. Flower of sulphur and white Seed-O-San decreased the percentage of germination for all three varieties of wheat. Copper carbonate did not affect the germination of durum wheat, but it probably increased that of Marquis.

TABLE XLIII

EFFECT OF TREATMENT WITH VARIOUS CHEMICALS ON THE PERCENTAGE OF GERMINATION OF WHEAT SEED

Treatment	Percentage of germination*									
	Marquis			Monad			Pentad			Av. of 3 varieties
	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	
Dusts										
Check—none	72	68	70.0	66	75	70.5	62	66	64.0	68.2
Nickel carbonate										
4 oz. per bu.	85	79	82.0	58	56	57.0	59	77	68.0	69.0
Copper carbonate										
4 oz. per bu.	90	90	90.0	83	81	82.0	81	78	79.5	83.8
Copper sulphate and										
lime, 4 oz. per bu.	80	85	82.5	75	66	70.5	74	68	71.0	74.6
Seed-O-San (pink)										
3 oz. per bu.	95	95	95.0	84	74	79.0	83	77	80.0	84.7
Seed-O-San (white)										
4 oz. per bu.	65	60	62.5	62	73	67.5	63	78	70.5	66.8
Flower of sulphur										
4 oz. per bu.	84	81	82.5	47	42	44.5	41	43	42.0	56.3
Liquids										
Chlorophol 0.25%										
soaked 2 hrs. cold	99	91	95.0	92	88	90.0	94	93	93.5	92.8
Tillantin "C" 0.25%										
soaked 2 hrs. cold	93	95	94.0	80	75	77.5	95	97	96.0	89.2
Semesan 0.3%										
soaked 2 hrs. ...	89	96	92.5	92	92	92.0	96	98	97.0	93.8
Silver nitrate n/100										
soaked 2 hrs.† ...	91	95	93.0	90	90	90.0	88	96	92.0	91.7
Germisan. 0.25%										
soaked ½ hr. cold	..	85	85.0	78	76	77.0	85	76	80.5	80.8
Germisan 0.25%										
soaked 1½ hrs. cold	82	81	81.5	89	84	86.5	84	89 .	86.5	84.8
Uspulun 0.25%										
soaked 1 hr. cold	88	89	88.5	88	89	88.5	91	84	87.5	88.2
Uspulun 0.25%										
soaked 2 hrs. cold	90	87	88.5	93	85	89.0	93	98	95.5	91.0
Uspulun 0.25%										
soaked ½ hr.										
40-45° C.	92	93	92.5	88	93	90.5	94	92	93.0	92.0
Uspulun 0.25%										
soaked 1½ hrs.										
40-45° C.	94	91	92.5	94	100	97.0	95	92	93.5	94.3

* Count in each series based on 100 seeds.

† Treated in all cases as cited in reference (24).

TABLE XLIV

EFFECT OF TREATMENT WITH VARIOUS CHEMICALS ON PERCENTAGE OF GERMINATION OF BARLEY SEED

Treatment	Percentage of germination*												Av. of 4 varieties
	Manchuria			Lion			Arequipa			Svansota			
	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.	
Dusts													
Check—none	90	92	91.0	98	87	92.5	87	90	88.5	87	87	87.0	89.7
Nickel carbonate, 4 oz. per bu.	90	89	89.5	100	82	91.0	84	99	91.5	92	94	93.0	91.2
Copper carbonate, 4 oz. per bu.	88	..	88.0	91	80	85.5	80	90	85.0	89	87	88.0	86.6
Copper sulphate and lime, 4 oz. per bu.	91	92	91.5	94	92	93.0	84	78	81.0	83	91	87.0	88.1
Seed-O-San (pink), 3 oz. per bu.	90	99	94.5	93	89	91.0	90	90	90.0	94	94	94.0	92.4
Seed-O-San (white), 4 oz. per bu.	97	97	97.0	90	90	90.0	87	79	83.0	100	93	96.5	91.6
Flower of sulphur, 4 oz. per bu.	85	92	88.5	95	85	90.0	80	82	81.0	88	93	90.5	87.5
Liquids													
Chlorophol 0.25% soaked 2 hrs.	92	100	96.0	99	91	95.0	97	92	94.5	99	98	98.5	96.0
Tillantin "C" 0.25% soaked 2 hrs.	94	97	95.5	94	92	93.0	92	97	94.5	91	96	93.5	94.1
Semesan 0.3% cold soaked 2 hrs.	87	97	92.0	95	96	95.5	94	94	94.0	97	95	96.0	94.4
Silver nitrate cold n/100 soaked 2 hrs.	98	94	96.0	91	90	90.5	93	99	95.5	100	99	99.5	95.4
Germisan 0.25% cold soaked ½ hr.	94	92	93.0	94	95	94.5	92	89	90.5	90	89	89.5	91.9
Germisan 0.25% cold soaked 1½ hrs.	93	94	93.5	92	90	91.0	87	100	93.5	94	93	93.5	92.9
Uspulun 0.25% cold soaked 1 hr.	93	96	94.5	100	91	95.5	95	96	95.5	99	98	98.5	96.0
Uspulun 0.25% cold soaked 2 hrs.	100	95	97.5	93	95	94.0	100	100	100.0	98	99	98.5	97.5
Uspulun 0.25% soaked ½ hr. 40-45° C.	97	98	97.5	100	92	96.0	94	97	95.5	95	94	94.5	95.9
Uspulun 0.25% soaked 1½ hrs. 40-45° C. ..	96	97	96.5	91	96	93.5	97	94	95.5	96	96	96.0	95.4

* One hundred seeds planted in each series.

The results of treating barley seed, presented in Table XLIV, are not so striking as those given in Table XLIII for wheat. In general, the relative percentage of germination of barley for the various treatments was similar to that noted for wheat. Apparently certain chemicals stimulate while others repress the percentage of germination of cereal seeds. The percentage of germination of untreated barley was much higher than that of untreated wheat, consequently there was less chance for stimulation in the barley than in the wheat; but the various fungicides seemed to affect both cereals similarly.

Increased percentage of germination has been attributed to the stimulatory effect of fungicides on seed and their effectiveness in controlling soil fungi. Many of the reports are conflicting. For this reason seeds of Mariout barley severely infected with *H. sativum* were treated with several fungicides. The treatments used are those listed in Table XLV. For each treatment 40 seeds were used. Twenty of these were plated out on two petri dishes of potato dextrose agar. The same number were sown in petri dishes containing moist sterilized white sand. For dust treatments, the seeds were rolled in an excess of the chemicals. Often considerable amounts adhered to the seeds after they were transferred to the plates.

TABLE XLV

Treatment	Percentage of colonies developed on potato dextrose agar		Percentage of seeds and seedlings on which *Helminthosporium* developed in sand	
	Helminthosporium sativum	Other fungi	Seed	Shoots
Dusts				
Check—none	85	15	55	100
Nickel carbonate, 4 oz. per bu.	90	10	65	100
Copper sulphate and lime, 4 oz. per bu....	90	10	75	100
Copper carbonate, 4 oz. per bu.	95	5	90	100
Seed-O-San (pink), 3 oz. per bu.	65	10	45	90
Seed-O-San (white), 4 oz. per bu.	95	5	70	100
Sulphur dust, 4 oz. per bu.	95	5	80	100
Liquids				
Chlorophol 0.25% soaked 2 hrs. cold	0	5	0	100
Tillantin "C" 0.25% soaked 2 hrs. cold....	10	45	30	80
Semesan 0.3% cold soaked 2 hrs.	80	10	35	100
Silver nitrate cold n/100 soaked 2 hrs......	70	10	0	80
Germisan 0.25% cold soaked ½ hr.	55	35	..	..
Germisan 0.25% cold soaked 1½ hrs.	60	25	..	..

The data in Table XLV show that none of the dust compounds killed the fungi on or in the seed. *H. sativum* and other fungi sporulated profusely on seed thus treated (Plate XII). Some liquid fungicides partially controlled *Helminthosporium*. Of these, Chlorophol and Tillantin were the most effective, but even these compounds merely

retarded the development of the pathogene. Eventually, in the sand cultures, discoloration became conspicuous and sporulation abundant on the basal portion of the young seedlings. These results indicate that the increase in percentage of germination with the dusts used can not always be explained on the basis of killing of the fungi within the seed. On the other hand, the results with a few of the liquid treatments seem to indicate that the chemicals retard the development of the pathogene in the seed and consequently lessen its injurious effect on seed germination.

The conflicting opinions regarding the effectiveness of seed disinfection can be explained to a certain extent. Various workers have used different varieties of cereals which were infected to different degrees. In 1923 Atanasoff (3) concluded that fungicides can not free internally-infested kernels from *Fusarium spp.* without killing the kernels. Drechsler (26) stated that preliminary treatments of badly discolored wheat seed with certain organic mercury compounds indicated control of seedling blight. Atanasoff and Johnson (4) have shown that *H. sativum* was practically eliminated from badly infested seeds of Chevalier barley by means of dry-heat treatment. They also were able to eliminate the wheat scab organism from several varieties of wheat in the same way. It will be recalled that the writer was unable to eliminate *H. sativum* completely from badly infected wheat and barley seed by the Jensen hot-water treatment.

EFFECT OF SEED TREATMENT ON GROWTH OF SEEDLINGS

It seems obvious that anything which stimulates the growth of seedlings would reduce the damage from the disease and vice versa. Numerous investigators have reported stimulation of seedling growth as a result of seed treatment. Mackie and Briggs (46) state that treatment of wheat seed with copper sulphate and formaldehyde represses growth, but that the application of copper carbonate dust to wheat seed results in vigorous seedlings. Stakman and Lambert (55), Heald and Smith (35), and others have reported stimulatory growth as the result of seed treatment.

In the present studies, observations were made on seedlings in the field and in the greenhouse to ascertain if seed treatment stimulated growth. The treatments and varieties of cereal used were the same as those used in the germination tests (Tables XLIII and XLIV).

None of the fungicidal dusts caused any appreciable stimulation of growth in the greenhouse. Several of the liquid compounds, especially Uspulun, Chlorophol, Tillantin, and silver nitrate, caused apparent stimulation, but the results were not conclusive. The stimulation was not always consistent in the different varieties and sometimes not even

in the two series of the same variety. This might be explained in part
by the higher percentage of germination due to the treatment, and the
consequent density of the stand. An increase in the percentage of
germination would naturally decrease the amount of space per plant.
On the other hand, copper sulphate, white Seed-O-San, and sulphur
dust consistently retarded the growth of the wheat seedlings in the
greenhouse, but not that of the barley. The plants in the plots treated
with the last-named compounds, as well as the controls, appeared to
be most severely attacked by root-rot. Within a month the basal por-
tion of all the plants in the experiment became infected conspicuously
with *Helminthosporium*. It will be recalled that the seeds were sown
in *Helminthosporium*-sick soil obtained from the field.

Observations on the different treatments in the field were made over
a period of two years. Each year the seed was sown in rod rows,
each row being replicated four times. There was no conclusive evi-
dence that fungicides stimulated or depressed seed germination and the
rate of growth of the seedlings. Either the treatments were not
effective, or the soil was so thoroly infested with *H. sativum* and other
root-rotting organisms as to obscure the effect of seed treatment.

The writer's results, therefore, do not corroborate those of other
investigators. The conditions of the experiments probably account for
this fact. Undoubtedly the kind and variety of grain, soil conditions,
and meteorological conditions affect results considerably.

EFFECT OF SEED TREATMENT ON DEVELOPMENT OF BASAL STEM-ROT AND ROOT-ROT

Seed treatment with dusts and liquid fungicides did not control
secondary root-rot and basal stem-rot. In 1923 notes were taken on
the amount of root-rot and basal stem-rot on 3 varieties of wheat and
3 of barley treated with the fungicides, except Germisan and Uspulun,
listed in Table XLVI. In 1924 observations were made for all the 16
different treatments. The varieties were grown in quadruplicated rod
rows. In neither season was there an appreciable difference in the
amount of root-rot and basal stem-rot in the different plots. The slight
variations which did occur were not consistent between the varieties,
and the variation between the replicated rows was as great as that
between any two treatments. The controls always were as free from
disease as the plants in the treated plots.

TABLE XLVI

EFFECTIVENESS OF SEED TREATMENTS IN PREVENTING KILLING OF MONAD AND PENTAD WHEATS BY ROOT-ROT AND BASAL STEM-ROT, AND A STATISTICAL COMPARISON WITH THE CONTROLS

Treatment	Av. per cent killed*	Statistical data in comparison with check for							
		Monad, C.I. 3320				Pentad, C.I. 3322			
		M.D.	S.D.	Z	Odds	M.D.	S.D.	Z	Odds
Dusts									
Check—none	72.2	...	...	...	...	...	...	...	...
Nickel carbonate, 4 oz. per bu.	69.5	2.75	6.01	.45	3.0	.75	1.29	.58	4.1
Copper sulphate and lime, 4 oz. per bu.	68.2	4.0	2.51	1.59	27.7	4.0	2.12	1.88	41.3
Copper carbonate, 4 oz. per bu.	73.5	—1.25	8.84	.13	1.4	4.75	3.23	1.38	19.6
Seed-O-San (pink) 3 oz. per bu.	72.0	0.25	4.96	.05	<1	11.0	6.20	1.77	35.5
Seed-O-San (white) 4 oz. per bu.	72.5	—0.25	7.69	.03	<1	6.25	6.58	.96	9.2
Sulphur dust, 4 oz. per bu.	71.0	1.25	4.65	.27	1.8	3.25	2.07	1.57	26.5
Liquids									
Chlorophol 0.25% soaked 2 hrs. cold........	75.0	—2.75	2.56	1.07	11.0	8.5	3.27	2.61	98.0
Tillantin "C" 0.25% soaked 2 hrs. cold	75.5	—3.25	6.37	.50	3.4	3.0	4.74	.63	4.6
Semesan 0.3% cold soaked 2 hrs.	71.0	1.25	8.07	.15	1.4	2.5	4.21	.59	4.2
Silver nitrate cold n/100 soaked 2 hrs.	75.0	—2.75	6.41	.42	2.7	2.0	1.87	1.07	11.4
Germisan 0.25% soaked ½ hr. cold	73.7	—1.50	9.75	.15	1.4	4.0	6.67	.59	4.2
Germisan 0.25% cold soaked 1½ brs.........	79.7	—7.50	2.10	3.02	38.3	4.0	6.81	.58	4.0
Uspulun 0.25% cold soaked 1 hr.	70.7	1.50	7.12	.23	1.7	4.5	1.50	3.00	144.0
Uspulun 0.25% cold snaked 2 hrs.	73.0	—0.75	6.82	.10	1.2	5.75	2.38	2.41	78.5
Uspulun 0.25% soaked ½ hr. 40-45° C.	76.2	—4.0	1.64	2.44	80.9	11.75	3.02	3.89	299.0
Uspulum 0.25% soaked 1½ hrs. 40-45° C.	76.5	—4.25	9.41	.45	3.0	7.25	6.64	1.09	11.9

* Average per cent of plants killed after heading, based on counting the number of plants in 4 replicated rod rows.

The percentage of Monad and Pentad wheat plants killed after heading in 1924 was determined by counting the total number of diseased and normal plants in the four replicated rod rows. The results of these counts are given in Table XLVI. The significance of these percentages was determined by "Student's Method" (64 and 65). The odds were obtained directly by looking up the value of Z in the table cited by Love (44) in the Journal of the American Society of Agronomy, Vol. 16, pp. 70-71, 1924. Certainly none of the treatments decreased or increased the percentage of Monad plants killed as compared with the controls. For Pentad, however, odds as high as 299 to 1 were secured in favor of treatment with Uspulun (soaked one half hour at 45° to 50° C.). In five treatments the odds were greater than 30 to 1 in favor of the treatments, but, owing to the lack of consistency between the two varieties of wheat, the results can not be considered conclusive.

EFFECT OF SEED TREATMENT ON YIELDS

In order to determine whether seed treatment would increase yields in the absence of smut, the following experiment was made. Three varieties of wheat—Marquis, Monad, and Pentad—and three varieties of barley—Manchuria, Lion, and Svansota—were treated as indicated in Tables XLVII and XLVIII and sown in *Helminthosporium*-sick soil. All fungicides except Germisan and Uspulun were used in both 1923 and 1924. Yield data were obtained from four systematically replicated rod rows (Tables XLVII and XLVIII). In general, seed treatment did not increase yields. In only 1 test out of 152 were the odds 30 to 1 or greater that the treatment increased yield. In 9 tests they were 30 to 1 or greater that the treatment had decreased the yield. The significance of the results was determined by "Student's Method." In the remaining 142 tests the results were not statistically significant. It is doubtful, therefore, whether the seed treatments used increased yields by decreasing the amount of infection by *H. sativum*.

TABLE XLVII

EFFECT OF VARIOUS FUNGICIDES ON YIELD OF BARLEY GROWN ON *Helminthosporium*-SICK SOIL AT UNIVERSITY FARM, ST. PAUL, IN 1923 AND 1924

	Manchuria (Minn. No. 184)									
Treatment	1923					1924				
	Av. yield, bu.*	M.D.	S.D.	Z	Odds	Av. yield, bu.*	M.D.	S.D.	Z	Odds
Dusts										
Check—none	34.8	...	...	...	...	37.6	...	...	...	...
Nickel carbonate, 4 oz. per bu.	32.8	−2.0	8.88	0.23	1.8	34.1	−3.52	3.44	1.02	10.0
Copper sulphate and lime, 4 oz. per bu.	28.3	−6.5	3.89	2.25	65.2	34.5	−3.05	3.50	0.87	7.4
Copper carbonate, 4 oz. per bu.	32.4	−2.35	4.99	0.47	3.0	36.6	−1.00	3.32	0.30	2.1
Seed O-San (pink) 3 oz. per bu.	29.1	−5.65	2.46	1.63	29.8	37.9	0.35	3.0	0.11	<1.0
Seed O-San (white) 4 oz. per bu.	36.0	1.2	12.45	0.08	<1.0	35.6	−1.90	2.80	6.80	5.4
Sulphur dust, 4 oz. per bu.	36.3	1.5	1.46	1.03	11.0	39.0	1.43	3.38	0.42	2.7
Liquids										
Chlorophol 0.25% soaked 2 hrs. cold	29.4	−5.4	6.32	0.85	7.4	42.4	4.80	3.33	1.45	21.9
Tillantin "C" 0.25% soaked 2 hrs. cold......	21.9	−12.9	7.39	1.74	34.5	37.1	−0.45	4.89	0.09	<1.0
Semesan 0.3% soaked 2 hrs. cold	30.5	−4.3	3.50	1.23	15.7	32.9	−4.72	6.19	0.77	5.9
Silver nitrate cold n/100 soaked 2 hrs.	31.0	−3.8	9.89	0.38	2.7	35.9	−1.72	3.46	0.49	3.4
Germisan 0.25% soaked ½ hr. cold	...	...	...	...	...	34.6	−3.00	4.94	0.60	4.3
Germisan 0.25% soaked 1½ hrs. cold	...	...	...	...	...	34.3	−3.32	1.86	2.51	86.7
Uspulun 0.25% soaked 1 hr. cold	...	...	...	...	...	34.9	−2.75	5.79	0.47	3.0
Uspulun 0.25% soaked 2 hrs. cold	...	...	...	...	...	35.2	−2.40	3.80	0.64	4.8
Uspulun 0.25% soaked ½ hr. 40-45° C.	...	...	...	...	...	38.0	0.40	5.04	0.07	<1.0
Uspulun 0.25% soaked 1½ hrs. 40-45° C.	...	...	...	...	...	39.7	2.10	8.13	0.26	1.8

* Average for four systematically replicated rod rows.

TABLE XLVII—*Continued*

EFFECT OF VARIOUS FUNGICIDES ON YIELD OF BARLEY (GROWN ON *Helminthosporium*-SICK SOIL AT UNIVERSITY FARM, ST. PAUL, IN 1923 AND 1924)

Lion, C. I. 923

Treatment	1923					1924				
	Av. yield, bu.*	M.D.	S.D.	Z	Odds	Av. yield, bu.*	M.D.	S.D.	Z	Odds
Dusts										
Check—none	24.6	...	...	...	...	70.3	...	...	...	...
Nickel carbonate, 4 oz. per bu.	23.1	−1.55	2.16	0.71	5.4	69.5	−0.8	5.24	0.15	1.4
Copper sulphate and lime, 4 oz. per bu.	20.9	−3.65	5.29	0.68	5.4	68.2	−2.15	7.55	0.28	2.1
Copper carbonate, 4 oz. per bu.	24.4	−0.20	5.83	0.03	<1.0	67.5	−2.8	10.14	0.27	1.8
Seed-O-San (pink) 3 oz. per bu.	19.2	−5.40	3.46	1.56	25.7	69.6	−0.7	6.12	0.01	<1.0
Seed-O-San (white) 4 oz. per bu.	23.1	−1.50	5.90	0.27	1.8	59.5	−11.9	12.6	0.94	9.0
Sulphur dust, 4 oz. per bu.	23.4	−1.20	4.66	0.25	1.8	67.1	−3.2	7.43	0.43	3.0
Liquids										
Chlorophol 0.25% soaked 2 hrs. cold	22.4	−1.95	4.03	0.48	3.4	66.1	−4.2	4.9	0.86	7.3
Tillantin "C" 0.25% soaked 2 hrs. cold	21.2	−3.40	4.83	0.70	5.4	59.9	−10.5	15.6	0.60	4.3
Semesan 0.3% soaked 2 hrs. cold	25.4	0.85	4.64	0.18	1.6	65.7	−4.6	6.12	0.75	5.9
Silver nitrate (old n/100 soaked 2 hrs.	20.7	−3.95	7.34	0.54	3.8	60.0	−10.3	9.69	1.06	11.0
Germisan 0.25% soaked ½ hr. cold	...	...	...	...	...	64.3	−6.05	13.3	0.45	3.0
Germisan 0.25% soaked 1½ hrs. cold	...	...	...	...	...	59.6	−10.8	9.5	1.14	13.2
Uspulun 0.25% soaked 1 hr. cold	...	...	...	...	...	65.1	−5.2	4.23	1.22	14.5
Uspulun 0.25% soaked 2 hrs. cold	...	...	...	...	...	70.6	0.25	3.9	0.06	<1.0
Uspulun 0.25% soaked ½ hr. 40-45° C.	...	...	...	...	...	72.9	2.55	3.2	0.80	6.7
Uspulun 0.25% soaked 1½ hrs. 40-45° C.	...	...	...	...	...	67.4	−2.9	4.2	0.69	5.4

* Average for four systematically replicated rod rows.

TABLE XLVII *Concluded*

EFFECT OF VARIOUS FUNGICIDES ON YIELD OF BARLEY GROWN ON *Helminthosporium*-SICK SOIL AT UNIVERSITY FARM, ST. PAUL, IN 1923 AND 1924

Treatment	Svansota (Minn. No. 440)									
	1923					1924				
	Av. yield, bu.*	M.D.	S.D.	Z	Odds	Av. yield, bu.*	M.D.	S.D.	Z	Odds
Dusts										
Check—none	33.2	...	...	...	...	39.6	...	...	...	...
Nickel carbonate, 4 oz. per bu.	30.1	−3.05	2.98	1.02	10.0	36.5	−3.07	6.2	0.49	3.4
Copper sulphate and lime, 4 oz. per bu.	26.1	−7.1	6.66	1.66	29.8	30.3	−9.35	6.32	1.48	23.9
Copper carbonate, 4 oz. per bu.	27.9	−5.3	2.82	1.88	42.5	38.6	−1.0	7.55	0.13	1.4
Seed-O-San (pink) 3 oz. per bu.	24.3	−8.95	6.89	1.29	17.2	37.0	−2.55	7.61	0.33	2.4
Seed-O-San (white) 4 oz. per bu.	...	...	...	...	...	37.3	−2.35	3.66	0.64	4.8
Sulphur dust, 4 oz. per bu.	...	...	...	...	...	37.9	−1.65	4.08	0.40	2.7
Liquids										
Chlorophol 0.25% soaked 2 hrs. cold	29.0	−4.22	7.72	0.55	3.8	38.3	−1.30	2.61	0.50	3.4
Tillantin "C" 0.25% soaked 2 hrs. cold	24.4	−8.8	4.04	2.18	61.9	36.7	−2.87	4.77	0.60	4.3
Semesan 0.3% soaked 2 hrs. cold	...	...	...	...	...	36.0	−3.62	5.01	0.72	5.4
Silver nitrate cold n/100 soaked 2 hrs.	...	...	...	...	...	37.6	−1.97	5.35	0.36	2.4
Germisan 0.25% soaked ½ hr. cold	...	...	...	...	...	37.7	−1.92	7.86	0.22	1.6
Germisan 0.25% soaked 1½ hrs. cold	...	...	...	...	...	37.7	−1.90	4.50	0.42	2.7
Uspulun 0.25% soaked 1 hr. cold	...	...	...	...	...	37.2	−2.40	2.23	1.02	10.0
Uspulun 0.25% soaked 2 hrs. cold	...	...	...	...	...	39.8	.12	3.30	0.03	1.0
Uspulun 0.25% soaked ½ hr. 40-45° C.	...	...	...	...	...	41.4	1.82	5.83	0.31	2.1
Uspulun 0.25% soaked 1½ hrs. 40-45° C. ...	...	...	...	...	...	35.2	−4.40	10.48	0.42	2.7

* Average for four systematically replicated rod rows.

TABLE XLVIII

EFFECT OF VARIOUS FUNGICIDES ON YIELD OF WHEAT GROWN ON *Helminthosporium*-SICK SOIL AT UNIVERSITY FARM, ST. PAUL, IN 1923 AND 1924

Marquis, C. I. 3641

Treatment	1923					1924				
	Av. yield, bu.*	M.D.	S.D.	Z	Odds	Av. yield, bu.*	M.D.	S.D.	Z	Odds
Dusts										
Check none	22.8	...	...	...	...	26.8	...	...	...	...
Nickel carbonate, 4 oz. per bu.	23.6	0.83	2.73	0.30	2.1	27.4	0.25	2.37	0.10	1.3
Copper sulphate and lime, 4 oz. per bu.	21.5	−1.25	1.31	.96	9.0	24.6	−2.20	1.21	1.91	47.7
Copper carbonate, 4 oz. per bu.	21.1	−1.68	3.39	.49	3.4	24.9	−1.90	2.06	0.92	8.2
Seed-O-San (pink) 3 oz. per bu.	25.0	2.25	5.69	.39	2.7	27.2	0.4	3.15	0.12	1.3
Seed-O-San (white) 4 oz. per bu.	22.7	−0.07	3.76	.01	<1.0	25.4	−1.4	3.46	0.40	2.7
Sulphur dust, 4 oz. per bu.	21.9	−0.90	2.03	.44	3.0	24.8	−2.0	.54	3.70	260.0
Liquids										
Chlorophol 0.25% soaked 2 hrs. cold	24.2	1.40	4.03	.33	2.4	25.4	−1.4	2.64	0.53	3.8
Tillantin "C" 0.25% soaked 2 hrs. cold	23.4	0.63	3.30	.19	1.6	26.6	−0.2	1.17	0.17	1.4
Semesan 0.3% soaked 2 hrs. cold	25.0	2.4	1.03	2.33	73.1	26.9	0.1	2.39	0.04	<1.0
Silver nitrate cold n/100 soaked 2 hrs.	22.0	−0.75	2.61	.28	2.1	26.0	−0.8	3.12	0.25	1.8
Germisan 0.25% soaked ½ hr. cold	...	...	...	...	...	27.3	0.55	4.97	0.11	1.3
Germisan 0.25% soaked 1½ hrs. cold	...	...	...	...	...	28.8	2.0	4.91	0.40	2.7
Uspulun 0.25% soaked ¼ hr. cold	...	...	...	...	...	25.8	−1.0	4.92	0.20	1.6
Uspulun 0.25% soaked 2 hrs. cold	...	...	...	...	...	26.3	−0.55	5.43	0.10	1.3
Uspulun 0.25% soaked ½ hr. 40-45° C.	...	...	...	...	...	32.1	5.30	9.08	0.58	4.3
Uspulun 0.25% soaked 1½ hrs. 40-45° C.	...	...	...	...	...	28.7	1.90	6.35	0.29	2.1

* Average for four systematically replicated rod rows.

TABLE XLVIII—*Continued*

Effect of Various Fungicides on Yield of Wheat Grown on *Helminthosporium*-Sick Soil at University Farm, St. Paul, in 1923 and 1924

Monad, C. I. 3320

Treatment	1923					1924				
	Av. yield, bu.*	M.D.	S.D.	Z	Odds	Av. yield, bu.*	M.D.	S.D.	Z	Odds
Dusts										
Check—none	17.0	...	...	...	...	14.1	...	...	...	...
Nickel carbonate, 4 oz. per bu.	18.4	1.35	4.37	0.38	2.7	13.9	—0.15	5.00	0.03	<1.0
Copper sulphate and lime, 4 oz. per bu.	16.2	—0.8	4.74	.17	1.4	17.1	3.0	3.40	0.88	8.2
Copper carbonate, 4 oz. per bu.	15.4	—1.6	6.01	.26	1.9	16.5	2.4	4.05	0.59	4.3
Seed-O-San (pink) 3 oz. per bu.	17.3	0.33	6.70	.04	<1.0	15.7	1.6	1.52	1.05	11.0
Seed-O-San (white) 4 oz. per bu.	15.7	—1.25	7.93	.16	1.4	13.8	—0.30	5.22	0.05	<1.0
Sulphur dust, 4 oz. per bu.	14.8	—2.20	3.45	.63	4.8	14.1	0.02	2.91	0.00	<1.0
Liquids										
Chlorophol 0.25% soaked 2 hrs. cold	19.8	2.80	2.93	.95	9.0	14.4	0.32	3.86	0.08	<1.0
Tillantin "C" 0.25% soaked 2 hrs. cold	17.4	0.38	6.55	.05	<1.0	13.1	—1.02	3.98	0.24	1.8
Semesan 0.3% soaked 2 hrs. cold	17.0	—0.02	4.84	.00	<1.0	14.9	0.85	2.07	0.41	2.7
Silver nitrate cold n/100 soaked 2 hrs.	16.7	—0.30	4.78	0.06	<1.0	13.7	—0.4	4.01	0.09	<1.0
Germisan 0.25% soaked ½ hr. cold	...	...	...	...	...	11.7	—2.35	3.94	0.62	4.3
Germisan 0.25% soaked 1½ hrs. cold	...	...	...	...	...	15.4	1.35	3.61	0.37	2.4
Uspulun 0.25% soaked 1 hr. cold	...	...	...	...	...	14.0	—0.10	3.43	0.03	<1.0
Uspulun 0.25% soaked 2 hrs. cold	...	...	...	...	...	15.8	1.70	6.79	0.25	1.8
Uspulun 0.25% soaked ½ hr. 40-45° C.	...	...	...	...	...	16.1	2.0	3.63	0.59	4.3
Uspulun 0.25% soaked 1½ hrs. 40-45° C.	...	...	...	...	...	14.8	0.75	3.99	0.19	1.6

* Average for four systematically replicated rod rows.

TABLE XLVIII *Concluded*

EFFECT OF VARIOUS FUNGICIDES ON YIELD OF WHEAT GROWN ON *Helminthosporium*-SICK SOIL AT UNIVERSITY FARM, ST. PAUL, IN 1923 AND 1924

Treatment	1923					Pentad, C. I. 332² 1924				
	Av. yield, bu.*	M.D.	S.D.	Z	Odds	Av. yield, bu.*	M.D.	S.D.	Z	Odds
Dusts										
Check—none	19.9	...	...	...	...	20.7	...	...	...	...
Nickel carbonate, 4 oz. per bu.	18.7	−1.15	3.44	0.33	2.4	19.7	−1.05	3.46	0.30	2.1
Copper sulphate and lime, 4 oz. per bu.	19.9	0.0	2.58	0.0	<1.0	21.4	0.70	2.30	.30	2.1
Copper carbonate, 4 oz. per bu.	22.3	2.45	2.29	1.07	11.0	20.5	−0.18	9.50	.01	<1.0
Seed-O-San (pink) 3 oz. per bu.	20.8†	2.30	1.93	1.19†	7.1†	22.3	1.60	3.49	.46	3.0
Seed-O-San (white) 4 oz. per bu.	19.6	−0.23	5.73	0.04	<1.0	23.2	2.50	3.80	.66	4.8
Sulphur dust, 4 oz. per bu.	22.1	2.20	3.84	0.58	4.3	21.4	0.72	3.10	.23	1.8
Liquids										
Chlorophol 0.25% soaked 2 hrs. cold	20.2	0.39	5.91	0.06	<1.0	23.7	−3.0	3.88	.72	5.4
Tillantin "C" 0.25% soaked 2 hrs. cold	20.3	0.45	2.08	0.21	1.6	19.2	−1.45	3.41	.42	2.7
Semesan 0.3% soaked 2 hrs. cold	18.9	−0.85	3.20	0.25	1.8	21.4	0.70	3.99	.17	1.4
Silver nitrate cold n/100 soaked 2 hrs.	17.5	−2.45	1.28	1.91	42.5	20.5	−0.23	1.24	.18	1.6
Germisan 0.25% soaked ½ hr. cold	...	...	...	...	...	21.4	0.72	4.13	.17	1.4
Germisan 0.25% soaked 1½ hrs. cold	...	...	...	...	...	21.8	1.1	4.81	.22	1.6
Uspulun 0.25% soaked 1 hr. cold	...	...	...	...	...	23.4	2.65	5.01	.52	3.4
Uspulun 0.25% soaked 2 hrs. cold	...	...	...	...	...	19.2	−1.45	4.38	.30	2.1
Uspulun 0.25% soaked ½ hr. 40-45° C.	...	...	...	...	...	22.3	1.58	2.36	.67	4.8
Uspulun 0.25% soaked 1½ hrs. 40-45° C.	...	...	...	...	...	20.8	0.05	2.57	0.01	<1.0

* Average for four systematically replicated rod rows.

† Only three replicated rod rows.

VARIETAL RESISTANCE

The study of varietal resistance is of paramount importance, since the disease can not be effectively controlled by seed treatment or cultural practices. In 1921 Hayes and Stakman (32) demonstrated that there were great differences between the resistance of barley varieties and purified hybrids to *H. sativum*. They concluded that there was no apparent correlation between botanical characters and resistance. In 1923 Hayes, Stakman, et al. (33) verified and extended these results. They obtained a white, resistant, smooth-awned barley from a cross between a black smooth-awned, susceptible and a resistant, rough-awned, six-rowed barley. They showed that the four species of *Hordeum—H. vulgare, H. intermedium, H. distichum,* and *H. deficiens*—contain varieties susceptible to *Helminthosporium*. All the species except *H. intermedium* also contained resistant varieties. Griffee (30) crossed Svansota and Lion and showed that there was partial linkage between white, two-rowed, rough-awned, and resistance factors. His results indicate that at least three factors or groups of factors were involved in determining the mode of inheritance of resistance or susceptibility.

Stakman (60), in 1919, was the first to report varietal differences in susceptibility to *Helminthosporium* in wheat. Aamodt (1) noted that in a cross between Marquis, a susceptible host, and Kanred, a moderately resistant variety, spring varieties with a high degree of resistance to root-rot were obtained. Christensen (18) observed varietal differences in susceptibility to root-rot in greenhouse tests. Stakman, Lambert, and Flor (56) reported variation in susceptibility for different species groups of wheat. Christensen and Stakman (20) recently called attention to the great susceptibility of durums to basal stem-rot.

Practically no work has been done on varietal resistance of wheat to *H. sativum*. For this reason, a preliminary survey of varietal susceptibility was made. The varieties used were obtained from the Office of Cereal Investigations, United States Department of Agriculture. For field tests, seven-foot rows were sown at the rate of six and a half grams of seed per row. At University Farm, St. Paul, the seed was sown in root-rot-sick soil. At Coon Creek, Minn., as well as at University Farm, the soil was inoculated on the date of seeding. The inoculum was grown on sterilized wheat and oat kernels and this was applied to the open rows before covering them. In 1923 a single row of each variety was grown at Coon Creek and at University Farm, while in 1924 duplicated rows were sown at both places. In both years notes were taken on the degree of root- and basal stem-rot and severity of seed blighting. In 1924 notes were also taken on the percentage

of plants killed after heading. As previously mentioned, the degree of infection for the purpose of exact comparison is designated by numerical figures. High figures, of which 13 is the maximum, denote complete susceptibility; while 1 signifies minimum infection observed. The results are presented in Table XLIX.

All varieties of *Triticum vulgare—T. compactum, T. durum, T. dicoccum, T. turgidum, T. spelta,* and *T. polonicum*—became infected with root- and basal stem-rot to a greater or lesser degree. The club wheats as a group appeared to be the most resistant, altho this apparent resistance may be due to late maturity. The durums were the most susceptible. There were striking differences in varietal susceptibility among the 73 varieties of common wheat, altho in general they were much more resistant than the durums. Only 2 varieties of *Triticum turgidum* were sown: Clackamas, C. I. 6241; and Alaska, C. I. 5988. The former was extremely susceptible to root-rot, the latter moderately so.

In general, the varieties which were resistant to root- and basal stem-rot at University Farm were resistant also at Coon Creek. Likewise, the varieties susceptible or resistant one year tended to be respectively susceptible or resistant the next year.

The results summarized in Tables L and LI show a close correlation between the behavior of two different series of wheat varieties to root-rot and basal stem-rot grown at Coon Creek and at University Farm. The coefficients were respectively $+0.6829 \pm 0.0352$ and $+0.4856 \pm 0.0505$.

Positive correlations were likewise obtained between the behavior of the varieties to root- and basal stem-rot at Coon Creek and University Farm in 1923 and 1924, and between the averages for the two years for the two localities.

The data are given in Tables LII to LIV inclusive. The results were respectively $+0.5123 \pm 0.0493$, $+0.4781 \pm 0.0512$, and $+0.4367 \pm 0.0557$. These coefficients indicate that susceptibility of wheat varieties to root- and basal stem-rot are inherited characters and not variations due to fluctuations in environmental conditions.

In order to determine whether differences in percentage of killing in 1924 were due to genetic or to ecological factors, the behavior of each variety in duplicate series was studied. The plants were grouped into classes, each class consisting of a 10 per cent range. A correlation coefficient of $+0.9760 \pm 0.0031$ was obtained, showing a practical identity in the reaction of each variety to basal stem-rot in the two series, thus again indicating that resistance to this malady is apparently a genetic character.

TABLE XLIX

Degree of Root- and Basal Stem Rot, and Seed Blight of 195 Varieties of Wheat Grown on Sandy Soil at Coon Creek and on Heavy Soil at University Farm in 1923 and 1924

Species and varieties	C.I. No.	Root- and basal stem-rot 1923 Univ. Farm	Coon Creek	Av.	1924 Univ. Farm Ser. A	Ser. B	Av.	Coon Creek Ser. A	Ser. B	Av.	Av. for two stations	Seed infection 1923 Univ. Farm	Coon Creek	1924 Univ. Farm	Coon Creek	Percentage killed after heading 1924 Univ. Farm Ser. A	Ser. B	Av.
Triticum vulgare																		
Early Defiance	6480	6	10	8	8	5	7	7	10	9	8	4	1	1	1	8	5	6.5
Colorado No. 50	4959	7	11	9	8	7	8	7	8	8	8	10	8	2	1	11	18	14.5
Touse	6047	5	9	7	6	6	6	6	7	7	7	3	3	2	1	17	16	16.5
Defiance	6477	7	7	7	7	7	7	6	9	8	8	1	2	2	1	8	13	10.5
Rink	5868	6	10	8	6	7	6	5	6	6	7	3	3	1	1	8	7	7.5
Bunyip	5125	7	10	8	6	6	6	5	7	6	6	2	4	3	1	23	20	21.5
Pacific Bluestem	4067	7	5	6	6	6	6	5	6	6	6	1	..	2	1	15	11	13.0
Mexican Bluestem	6004	7	8	8	8	6	7	6	6	6	6	3	1	3	1	20	29	24.5
Dart	5128	7	9	8	8	6	7	6	7	7	7	3	..	1	1	6	13	9.5
Gypsum	4762	8	10	9	6	7	6	6	7	7	7	3	4	2	1	12	15	13.5
Surprise	2986	8	10	9	6	6	6	6	7	7	7	7	4	2	1	17	15	16.0
Dicklow	3663	8	10	9	9	9	9	6	7	7	8	3	3	1	2	22	16	19.0
Quality	6157	7	10	8	9	8	9	8	10	9	9	5	3	2	2	8	6	7.0
Bobs	4991	9	12	11	9	6	8	7	6	6	8	8	5	1	1	12	19	15.5
White Fife	4412	7	10	8	5	5	5	8	7	7	7	1	1	3	1	3	3	3.0
White Federation	4981	8	10	9	6	7	7	7	6	7	7	5	7	1	1	5	23	14.0
Lynn	6346	8	8	8	5	6	5	8	7	7	7	5	7	3	1	6	6	6.0
Regenerated Defiance	3703	6	9	8	6	5	6	7	6	6	6	9	1	2	1	2	3	2.5
New Zealand	6011	6	6	6	8	7	7	6	6	6	7	2	1	1	1	7	2	4.5
Pilcraw	5540	6	9	8	5	7	6	6	6	6	7	2	1	1	1	14	11	12.5
Kinney	5189	5	5	5	5	3	4	5	9	7	6	1	..	2	1	8	10	9.0
Purple Straw	1915	5	8	7	5	5	5	5	7	6	6	1	..	1	1	17	14	15.5
Huston	5208	6	7	7	6	7	7	6	8	8	8	4	3	3	..	4	9	6.5

* Symbols of infection —0=Absence of infection, 1=Trace—, 2=Trace—, 3=Trace, 4=Trace+, 5=Light, 6=Light+, 7=Moderate—, 8=Moderate, 9=Moderate+, 10=Heavy—, 11=Heavy—, 12=Heavy+.

TABLE XLIX—Continued

DEGREE OF ROOT- AND BASAL STEM-ROT, AND SEED BLIGHT OF 105 VARIETIES OF WHEAT GROWN ON SANDY SOIL AT COON CREEK AND ON HEAVY SOIL AT UNIVERSITY FARM IN 1923 AND 1924

Species and varieties	C.I. No.	Root- and basal stem-rot										Seed infection				Percentage killed after heading		
		1923			1924						Av. for two stations	1923		1924		1924 Univ. Farm		
		Univ. Farm	Coon Creek	Av.	Univ. Farm			Coon Creek				Univ. Farm	Coon Creek	Univ. Farm	Coon Creek			
					Ser. A	Ser. B	Av.	Ser. A	Ser. B	Av.						Ser. A	Ser. B	Av.
Red Bobs	6255	7	11	9	8	7	8	7	7	7	8	1	2	1	1	10	13	11.5
Marquis	3641	5	11	8	6	9	8	5	8	7	8	3	4	1	1	6	7	6.5
Red Fife	3329	6	8	7	6	8	7	5	8	7	7	1	1	1	1	8	11	9.5
Power	3697	7	10	9	6	6	6	5	8	7	7	2	1	3	1	6	8	7.0
Glyndon	2870	8	10	9	6	7	7	5	7	6	7	1	2	1	1	6	6	6.0
Rysting	3022	8	9	9	8	6	7	5	7	6	7	1	5	3	1	8	5	6.5
Wellman	4144	7	8	8	7	7	7	5	7	6	7	5	3	3	1	9	10	9.5
Early Red Fife	4932	8	8	8	5	6	7	6	7	7	7	1	4	1	1	5	11	8.0
Ghirka	1517	8	8	8	5	6	6	6	7	7	7	3	2	1	1	5	2	3.5
Ruby	6047	9	11	10	9	8	9	7	7	7	7	5	1	2	1	4	3	3.5
Kitchener	4800	7	9	8	8	7	7	6	7	7	7	3	1	2	1	19	15	17.0
John Brown	4121	7	8	8	8	6	7	8	8	8	8	3	3	2	1	10	8	9.0
Allen	5407	6	7	7	7	7	7	7	7	7	7	3	3	4	1	10	8	9.0
Federation	4734	4	7	6	7	9	8	7	7	7	8	2	2	1	1	27	18	22.5
Foisy	5242	5	6	6	7	6	7	5	5	5	6	3	3	6	1	8	8	8.0
Schlanstedt	4646	4	5	5	6	5	6	5	6	6	6	3	4	1	··	5	3	4.0
Hard Federation	4980	7	11	9	10	9	10	10	9	9	10	1	··	1	1	24	24	24.0
Resaca	6390	7	5	6	7	8	8	5	6	6	7	1	··	1	2	3	4	3.5
Stanley	4796	6	9	8	7	6	7	6	6	6	7	1	1	1	1	17	7	12.0
Jumbuck	4608	6	9	8	9	8	8	7	8	8	8	5	··	2	1	22	14	18.0
Indian	4489	9	11	10	7	6	7	5	6	6	8	5	8	3	1	11	9	10.0
Haynes Bluestem	2874	3	8	6	8	7	8	6	6	6	7	3	1	2	1	11	9	10.0
Dakota	3083	7	7	7	6	6	6	6	6	6	6	1	··	1	1	6	8	7.0
Galgalos	2398	9	10	10	7	8	8	7	8	8	8	2	2	2	1	20	14	17.0

* Symbols of infection—o=Absence of infection, 1=Trace—, 2=Trace, 3=Trace+, 4=Light—, 5=Light, 6=Light+, 7=Moderate—, 8=Moderate, 9=Moderate+, 10=Heavy—, 11=Heavy, 12=Heavy+.

TABLE XLIX (Continued)

DECREASE IN ROOT AND BASAL STEM-ROT, AND SEED BLIGHT OF 105 VARIETIES OF WHEAT GROWN ON SANDY SOIL AT COON CREEK AND ON HEAVY SOIL AT UNIVERSITY FARM IN 1923 AND 1924

Species and varieties	C. I. No.	Root- and basal stem-rot 1923 Univ. Farm	1923 Coon Creek	Av.	1924 Univ. Farm Ser. A	Ser. B	Av.	1924 Coon Creek Ser. A	Ser. B	Av.	Av. for two stations	Seed infection 1923 Univ. Farm	1923 Coon Creek	1924 Univ. Farm	1924 Coon Creek	Percentage killed after heading 1924 Univ. Farm Ser. A	Ser. B	Av.
Sonora	3036	9	12	11	5	8	7	7	8	8	8	8	6	2	1	11	9	10.0
Palisade	4798	6	9	8	7	7	7	9	7	8	8	8	1	1	1	17	10	13.5
Propo	1970	7	9	8	8	6	7	7	10	9	8	3	3	1	1	14	9	11.5
Baart	1697	8	11	10	9	8	9	7	6	7	8	4	1	3	.	30	14	22.0
Talimka	2495	9	12	11	10	8	9	10	10	10	10	3	3	6	1	8	6	7.0
Champlain	4782	6	10	8	6	8	8	7	7	7	8	1	2	.	1	7	7	7.0
Java	4966	7	12	10	9	8	9	7	10	9	9	3	1	1	1	12	20	16.0
Erivan	2397	9	12	11	10	8	9	8	10	9	9	6	2	1	1	4	3	3.5
Converse	4141	6	9	8	7	6	7	7	10	9	8	4	1	1	1	5	11	8.0
Preston	3328	7	9	8	6	6	6	5	7	6	6	3	.	1	1	3	6	4.5
Kota	5898	7	10	9	7	5	6	7	7	7	7	2	2	1	1	3	3	3.0
Pioneer	4324	8	11	10	8	6	7	9	10	10	9	4	2	1	1	5	4	4.5
Fretes	1596	6	8	7	6	6	6	6	8	7	7	6	3	2	1	8	10	9.0
Dixon	6049	7	7	8	5	5	5	6	6	6	6	1	2	1	1	7	10	8.5
Chul	2227	7	12	10	7	8	8	8	8	8	8	7	8	2	3	3	5	4.0
Emerald	4397	7	7	7	8	9	9	6	6	6	8	3	4	1	.	10	16	13.0
Canadian Red	6282	7	7	7	10	9	10	7	7	7	9	5	5	6	1	7	15	11.0
Sevier	6247	8	6	7	6	6	6	5	6	6	6	2	1	5	1	11	7	9.0
Huron	3315	7	8	8	6	5	6	6	7	7	7	1	1	1	1	3	12	7.5
Norka	4377	6	8	7	6	6	6	7	7	7	7	1	1	2	1	5	7	6.0
Ladoga	4795	6	7	7	6	5	6	7	7	7	7	2	1	2	1	3	6	4.5
Laramie	6235	6	5	6	8	6	7	8	7	7	7	2	1	1	1	4	5	4.5
Prelude	4323	10	8	9	10	9	10	9	9	9	10	1	1	2	1	3	7	5.0

* Symbols of infection—o=Absence of infection, 1=Trace—, 2=Trace, 3=Trace+, 4=Light—, 5=Light, 6=Light+, 7=Moderate—, 7=Moderate+, 8=Moderate, 9=Moderate+, 10=Heavy—, 11=Heavy, 12=Heavy+.

TABLE XLIX—*Continued*

DEGREE OF ROOT- AND BASAL STEM-ROT, AND SEED BLIGHT OF 105 VARIETIES OF WHEAT GROWN ON SANDY SOIL AT COON CREEK AND ON HEAVY SOIL AT UNIVERSITY FARM IN 1923 AND 1924

Species and varieties	C. I. No.	Root- and basal stem-rot — Degree of infection*										Seed infection				Percentage killed after heading		
		1923 Univ. Farm	1923 Coon Creek	Av.	1924 Univ. Farm Ser. A	Ser. B	Av.	1924 Coon Creek Ser. A	Ser. B	Av.	Av. for two stations	1923 Univ. Farm	1923 Coon Creek	1924 Univ. Farm	1924 Coon Creek	1924 Univ. Farm Ser. A	Ser. B	Av.
Humpback	3690	7	7	7	6	7	7	5	6	6	7	1	2	1	1	9	6	7.5
Marquis × Iumillo...	II-1544	8	..	..	10	7	9	7	8	8	9	2	..	1	1	13	18	15.5
Marquis × Kanred...	II-1557	8	..	..	7	6	7	7	7	7	7	2	..	1	1	6	5	5.5
Average																9.9	10.1	10.0
Triticum compactum																		
Little Club	4066	6	6	6	7	7	7	6	8	7	7	2	1	3	1	11	14	12.5
Big Club	4257	7	6	7	7	7	7	7	8	8	8	1	1	3	1	10	9	9.5
Hybrid 143	4160	5	5	5	9	7	8	5	8	8	8	1	..	2	..	12	15	13.5
Hybrid 63	4570	5	5	5	6	7	7	6	7	7	7	..	..	1	2	3	3	3.0
Hybrid 108	5025	5	5	5	6	7	7	6	7	7	7	3	..	1	1	4	4	4.0
Hybrid 123	4511	5	6	6	6	6	6	5	6	6	6	1	..	1	..	7	3	5.0
Jenkin	5177	6	6	6	5	6	6	6	7	7	7	1	..	2	..	7	6	6.5
Redchaff	4241	5	7	6	8	9	9	6	7	7	8	1	..	3	1	5	8	6.5
Bluechaff	5256	5	6	6	8	7	8	6	7	7	8	1	..	2	1	8	8	8.0
Dale	4155	5	6	6	8	7	8	5	8	8	8	1	..	1	1	5	4	4.5
Wilbur	6796	6	8	7	8	8	8	10	9	10	9	8	..	3	2	5	5	5.0
Mayview	5874	7	6	7	9	9	9	6	7	7	8	1	..	1	1	11	4	7.5
Average																7.3	6.9	7.1
Triticum turgidum																		
Clackamas	6241	6	9	8	12	12	12	8	10	9	11	6	..	..	3	87	90	88.5
Alaska	5988	8	6	7	9	7	8	8	10	9	9	1	..	2	3	15	16	15.5
Average																51.0	53.0	52.0

* Symbols of infection: o = Absence of infection, 1 = Trace—, 2 = Trace, 3 = Trace+, 4 = Light—, 5 = Light, 6 = Light+, 7 = Moderate—, 8 = Moderate, 9 = Moderate+, 10 = Heavy—, 11 = Heavy, 12 = Heavy+.

TABLE XLIX.- (concluded)

DEGREE OF ROOT AND BASAL STEM ROT, AND SEED BLIGHT OF 105 VARIETIES OF WHEAT GROWN ON SANDY SOIL AT COON CREEK AND ON HEAVY SOIL AT UNIVERSITY FARM IN 1923 AND 1924

Grouping of columns: **Degree of infection*** — *Root- and basal stem-rot:* 1923 (Univ. Farm, Coon Creek, Av.); 1924 Univ. Farm (Ser. A, Ser. B, Av.); 1924 Coon Creek (Ser. A, Ser. B, Av.); Av. for two stations. **Seed infection** — 1923 (Univ. Farm, Coon Creek); 1924 (Univ. Farm, Coon Creek). **Percentage killed after heading** — 1924 Univ. Farm (Ser. A, Ser. B, Av.).

Species and varieties	C. I. No.	1923 UF	1923 CC	1923 Av.	1924 UF Ser. A	1924 UF Ser. B	1924 UF Av.	1924 CC Ser. A	1924 CC Ser. B	1924 CC Av.	Av. two stations	Seed 1923 UF	Seed 1923 CC	Seed 1924 UF	Seed 1924 CC	% killed Ser. A	% killed Ser. B	% killed Av.
Triticum durum																		
Fentad	3322	9	9	9	11	8	10	9	9	9	10	9	10	3	3	86	74	80.0
Peliss	1584	8	9	9	10	9	10	9	9	9	10	10	7	2	2	83	90	86.5
Acme	5284	9	9	9	10	9	10	10	10	10	10	7	3	6	2	84	87	85.5
Monad	3320	9	9	9	12	9	11	9	9	9	10	5	9	3	2	80	79	79.5
Arnautka	1494	5	5	5	10	9	10	7	9	8	9	4	·	4	2	84	78	81.0
Mindum	5296	9	7	8	11	10	11	7	8	8	10	7	2	6	2	93	90	91.5
Kubanka	1440	9	6	8	11	10	11	9	8	9	10	5	9	3	2	89	74	81.5
Kubanka No. 8	4063	9	9	9	10	10	10	9	8	8	10	5	7	3	2	78	71	74.5
Buford	5395	8	10	9	11	8	10	9	7	8	9	5	7	3	1	82	83	82.5
Marcuani	2222	8	11	9	12	9	11	8	10	9	10	7	8	2	3	82	88	85.0
Velvet Don	1593	9	10	10	11	9	10	7	10	9	10	4	8	1	·	89	90	89.5
Golden Ball	6227	8	10	9	11	12	12	7	7	7	10	11	9	1	1	88	98	93.0
Kahla	5529	7	9	8	10	13	12	7	7	7	10	4	6	6	1	83	82	82.5
Iumillo selection		·	·	·	9	9	9	8	8	8	9	8	·	2	1	21	26	23.5
Average																80.1	79.3	79.7
Triticum dicoccum																		
Khapli	4013	11	13	12	12	12	12	10	11	11	12	·	·	·	·	·	·	·
Vernal	1524	5	7	6	12	7	10	6	8	7	9	·	·	1	1	20	13	16.5
Triticum spelta																		
White Spring	1775	6	6	6	9	5	7	5	9	7	7	·	·	1	1	24	16	20.0
Triticum polonicum																		
White Polish	3007	10	9	10	11	11	11	7	7	7	9	·	·	3	1	11	33	22.0
Average for total varieties																20.1	20.2	20.2

* Symbols of infection, o = Absence of infection, 1 = Trace—, 2 = Trace, 3 = Trace +, 4 = Light—, 5 = Light, 6 = Light+, 7 = Moderate—, 8 = Moderate, 9 = Moderate+, 10 = Heavy—, 11 = Heavy, 12 = Heavy+.

TABLE L

CORRELATION BETWEEN DEGREE OF ROOT-ROT AND BASAL STEM-ROT IN 105 VARIETIES OF WHEAT GROWN IN TWO DIFFERENT SERIES AT UNIVERSITY FARM IN 1924

Degree of infection in Series II

		3	4	5	6	7	8	9	10	11	12	13	
Degree of infection in Series I	5	1		4	2		1						8
	6			3	13	10	1	·	2				29
	7			1	4	8	3	1					17
	8			1	7	5	1	2					16
	9			1	1	2	5	3	·				12
	10					1	2	6	1			1	11
	11						2	1	2	1	1		7
	12					1		2			2		5
		1	0	10	27	27	15	17	3	1	3	1	105

$$r = +.6829 \pm .0352$$

TABLE LI

CORRELATION BETWEEN DEGREE OF ROOT-ROT AND BASAL STEM-ROT OF 105 VARIETIES OF WHEAT GROWN IN TWO DIFFERENT SERIES AT COON CREEK IN 1924

Degree of infection in Series II

		5	6	7	8	9	10	11	
Degree of infection in Series I	5	1·	8	5	5	2			21
	6		7	16	3	1			27
	7		6	12	8	1	5		32
	8			3	2		5		11
	9			2	3	3	2		10
	10			1		1	1	1	4
		1	22	39	21	8	13	1	105

$$r = +.4856 \pm .0505$$

An attempt was made to determine whether a correlation existed between the average percentage of plants killed in 1924 and the average degree of root- and basal stem-rot on 101 varieties of wheat grown at Coon Creek and University Farm in 1923. A correlation coefficient of $+0.4015 \pm 0.0560$ was obtained, which seems to show that such correlation possibly exists.

In both years separate notes were taken on the degree of discoloration of the seed. The results are presented in Table XLIX. There was considerable variation in degree of susceptibility between different varieties and species. The durums as a group were the most susceptible

varieties. In 1923 a correlation coefficient was computed for the degree of seed infection for 79 varieties of wheat grown at University Farm and at Coon Creek (Table LV). The correlation was +0.6738±0.0414. For the same year the correlation coefficient for seed infection of 102 varieties at University Farm as related to severity of basal stem-rot and root-rot was +0.4015±0.0560 (Table LVI). Thus the degree of seed infection appears to be related to varietal susceptibility and is also correlated with root infection. Seed discoloration may be used to denote a positive degree of susceptibility of a variety to root- and basal stem-rot, but a light seed discoloration does not necessarily mean a relatively weak root infection. This is apparent from Table XLIX. It will be observed that there was practically no seed discoloration in 1924, yet severe root-rot developed on many varieties.

TABLE LII

CORRELATION BETWEEN DEGREE OF ROOT- AND BASAL STEM-ROT IN 102 VARIETIES OF WHEAT GROWN AT COON CREEK AND UNIVERSITY FARM IN 1923

Degree of root- and basal stem-rot, Coon Creek

		5	6	7	8	9	10	11	12	13	
Degree of root- and basal stem-rot, University Farm	3				1						1
	4	1	1								2
	5	5	4	2	1	1		1			14
	6	1	4	3	4	7	3				22
	7	2	2	5	5	5	5	4	2		30
	8		2		2	3	7	2			16
	9		1	1		4	1	3	4		14
	10				1	1					2
	11									1	1
		9	13	12	14	21	16	10	6	1	102

r .5123 ± .0493

TABLE LIII

CORRELATION BETWEEN DEGREE OF ROOT- AND BASAL STEM-ROT OF 105 VARIETIES OF WHEAT GROWN AT COON CREEK AND UNIVERSITY FARM IN 1924

Degree of root- and basal stem-rot, Coon Creek

		5	6	7	8	9	10	11	
	4			1					1
	5		2	1	1				4
	6		8	10	1	1			20
Degree of root- and basal stem-rot, University Farm	7	1	7	14	7	3	1		33
	8		2	8	3	1	1		15
	9		1	6	2	3	1		13
	10			2	2	5	1		10
	11			1	1	3			5
	12			2		1		1	4
		1	20	45	17	17	4	1	105

$r = +.4781 \pm .0512$

TABLE LIV

CORRELATION BETWEEN AVERAGE DEGREE OF ROOT- AND BASAL STEM-ROT OF 102 VARIETIES OF WHEAT GROWN AT COON CREEK AND UNIVERSITY FARM IN 1923 AND 1924

Average degree of infection in 1924

		6	7	8	9	10	11	12	
	5	2	2	1	1				6
	6	3	9	4	1				17
	7	3	7	5	3				18
Average degree of infection in 1923	8	4	10	8	·1	3			26
	9	1	7	3	1	9			21
	10			5	3	1			9
	11			2	1	1			4
	12							1	1
		13	35	28	11	14	0	1	102

$r = .4367 \pm .0541$

TABLE LV

CORRELATION BETWEEN DEGREE OF SEED INFECTION IN 79 VARIETIES OF WHEAT GROWN AT
UNIVERSITY FARM AND COON CREEK IN 1923

Degree of infection at Coon Creek

Degree of infection at University Farm	1	2	3	4	5	6	7	8	9	10	
1	10	5		1	1						17
2	7	4		1							12
3	5	1	8	3							17
4	5	1	1			1		1			9
5			2		1		3	1	2		9
6		1	1								2
7		1	1	1				2			5
8				1	1	1					3
9							1			1	2
10							1	1			2
11									1		1
	27	13	13	7	3	2	5	5	3	1	79

$r = +.6738 \pm .0414$

TABLE LVI

CORRELATION COEFFICIENT BETWEEN SEVERITY OF BASAL STEM- AND ROOT-ROT AND SEED
INFECTION IN 102 VARIETIES OF WHEAT GROWN AT UNIVERSITY FARM IN 1923

Degree of seed infection in 1923

Degree of root infection in 1923	1	2	3	4	5	6	7	8	9	10	11	
3			1									1
4	1	1										2
5	8		5	1								14
6	7	5	3	3	1	2		1				22
7	12	3	7	1	3		1	1		1	1	30
8	3	2	3	3	2		1		1	1		16
9		11	1	1	4	1	3	2	1			14
10	1		1									2
11				1								1
	32	12	21	10	10	3	5	4	2	2	1	102

$r = +.4015 \pm .0560$

CONTROL MEASURES

The *Helminthosporium* disease of cereals is difficult to control. The pathogene lives over in and on the seed of several cereal crops and many wild and cultivated grasses. It develops saprophytically on plant remains in and on the surface of the soil and is an inter-crop parasite. The progress of the disease depends very largely on environmental conditions, but the pathogene is a virulent parasite under certain conditions.

Root-rot and basal stem-rot can not be controlled by planting clean seed in infested soil. However, the amount of seedling blight can be reduced by treating the seed with certain mercuric compounds, provided it is sown in comparatively clean soil. Such treatments also stimulate germination. They retard and partially eliminate primary infection and thus reduce the amount of inoculum early in the season, but they do not eliminate secondary infection and will not reduce the amount of blighting on foliage, spikes, and seed.

Rotation of crops is important in controlling root-rot and basal stem-rot. Observations indicate that the disease is more prevalent in fields which have been cropped continuously to wheat and barley than in those in which rotation has been practiced. As the organism over-winters in the soil, a rotation which includes immune or highly resistant crops such as corn, clover, timothy, or vegetables, is detrimental to the pathogene and consequently reduces the disease.

Early sowing of susceptible spring grains will aid somewhat in reducing the amount of seedling blight, because the optimum temperature for the cereal hosts is low while that for the pathogene is high. The time of sowing, however, does not affect the amount of spike, foliage, and seed blight. The destruction of susceptible grasses is important. The pathogene attacks at least 83 species of wild grasses belonging to 37 different genera (18). Many of these grasses are common weeds which produce a tremendous amount of inoculum.

Varietal resistence is of the utmost importance in controlling the disease, altho it must be remembered that resistance is only relative. The degree of infection is influenced profoundly by ecological conditions and cultural methods—rotation, etc. Velvet, Manchuria, and Svansota barleys (23) are resistant to *H. sativum*. The results of varietal tests with wheat indicate sufficient differences in susceptibility to make possible the selection and breeding of desirable resistant varieties.

DISCUSSION AND CONCLUSIONS

The disease of cereals caused by *Helminthosporium sativum* is prevalent and destructive in Minnesota every year; furthermore, it is widely distributed in the United States and other countries. Undoubtedly it causes great damage. The pathogene reduces the percentage of seed germination, causes seedling blight, kills older plants prematurely, infects the floral parts, and blights the seed, and often causes severe spotting of the foliage. In 1924, from 20 to 75 per cent of the plants of durum wheat in many fields in Minnesota were killed after they had headed. *H. sativum* was primarily responsible. On certain types of soil, especially peat, the disease is so destructive as to be a limiting factor in the production of wheat and barley.

H. sativum is a group species consisting of many physiologic forms. At least 37 can be recognized readily on culture media. The writer observed at least 50 distinct forms, and there are indications that there are numerous others. Eleven distinct forms were isolated from material collected in the vicinity of St. Paul alone. Most of the isolations from other regions were somewhat different.

Physiologic forms differ from each other strikingly on culture media in the following characters: habit of growth, rate of growth, amount of sporulation, zonation, color of mycelium, and temperature relations. The differences between forms sometimes are so great as to suggest the desirability of considering them as separate species.

The 37 forms on which an intensive study was made, differ from each other consistently even under identical conditions, and each form is very variable under different conditions. For this reason extreme caution is necessary in describing species, such as *H. sativum* and other fungi, which may vary equally much. Since a description of *H. sativum* based on the characters of all existing physiologic forms is quite impossible, the characters of at least a few of the forms should be taken into consideration.

Not only do the physiologic forms differ in general appearance on culture media, but they are strikingly different also in other respects. There is good preliminary evidence that different forms react quite differently to temperature. Most important of all, they differ pathogenically.

Many of the physiologic forms have quite different parasitic capabilities on wheat and barley. Some forms are extremely virulent, others are moderately virulent, and still others are only weak parasites. These differences in virulence of forms are of paramount importance, because they complicate the study of genetic inheritance and the development of resistant varieties. Varieties which are resistant in one region

may be very susceptible in another. Therefore a study of the number and parasitic capabilities of physiologic forms is prerequisite to sound procedure in breeding work. The problem is essentially local or, at best, regional. Results obtained in one locality may not be applicable in other localities. But this is not the worst feature of the situation. If one could be assured that these forms were relatively stable, the situation would not be especially bad. But they are not stable. Some of them are very unstable.

Mutations are extremely common on culture media. New forms are continually arising. Some forms mutate much more frequently than others. The mutants observed differed from the parents in rate of growth, color, zonation, amount of aerial mycelium, and pathogenicity. Relatively weak forms may produce very virulent mutants and vice versa. This change in parasitism is tremendously important. *H. sativum* is dynamic, not static. It is in a state of flux, and, consequently, presents a continually changing problem. There is every reason to suppose that mutation occurs in nature as well as on artificial media. A final solution of the problem is therefore extremely difficult.

The nuclear phenomena involved in the mutation of *H. sativum* are not known. The possibility of segregation as a result of nuclear fusion is not excluded, altho it does not seem to be very strong. Anastomosing hyphae have often been observed. Anastomosis between different types of *Helminthosporium* was noted by the writer. It is possible that hybridization may occur quite frequently. However, the mutants, which arise as sectors, suggest that the mechanism is somewhat analagous to bud mutations in higher plants.

The present studies have shown that the viability of the spores is greatly affected by ecological factors. The pathogene overwinters readily in the soil and in the seed. The organism is present in and on seed of resistant cereals. It also is a facultative saprophyte and grows on dead or dying plant remains. Primary infections may come from the seed or from the soil. High temperature and relatively high humidity are detrimental to the longevity of the spores, and lack of aeration results in their rapid death; but the conidia can withstand considerable alternation of temperature—thawing and freezing. They remain viable in the soil from one season to the next. It has been shown that the sowing of clean or treated seed does not insure plants free from infection if sown in infested soil.

The severity of disease is influenced profoundly by environmental conditions. The effect of moisture and temperature is twofold: the effect on the host and that on the pathogene. Factors such as soil fertility, physical characteristics, and chemical composition of the soil also influence the development of the disease. The maximum damage

from the disease results from conditions which are unfavorable for host development.

It is obvious that the control of *H. sativum* is difficult. The use of resistant varieties seems to be the most promising method of control. Resistant varieties of barley are known. Studies on varietal suscepti-bility of wheat to *H. sativum* indicate differences in resistance. Furthermore, the resistance is only relative. Extensive field experi-ments and observations for the last 5 years have shown that epidemics of *H. sativum* may occur in spite of the use of resistant varieties. It seems fair to conclude, therefore, that the use of resistant varieties must be in connection with clean seed, good cultural practice, and proper rotation of crops, in order to reduce the effects of the disease to a minimum.

SUMMARY

1. *Helminthosporium sativum* is widespread and destructive. It causes serious losses in Minnesota every year. It was isolated from material obtained in many states in the United States, from several places in Mexico and Canada, and from one place in Serbia.

2. *H. sativum* is a common inhabitant of cereal seeds. The amount present varies with the locality, year, and variety; but the percentage of infection is not actually correlated with the reaction of the plants in the field.

3. Over 1000 isolations were made from wheat plants affected with basal stem-rot. *H. sativum* was by far the most prevalent organism.

4. Zonation is fairly common but differs in the different forms. It apparently is caused by the amount and kind of medium and by alternating temperatures. Light is not essential, altho diurnal changes may induce zonation.

5. There are numerous physiologic forms of *H. sativum*. The writer studied 37 in detail. These forms can be distinguished in cul-ture by the following characters: rate of growth; proportion of sub-merged and aerial mycelium; nature of mycelial growth, such as zonation, production of conidia, density of conidial clusters, and color of the mycelium.

6. The forms also differ from each other pathogenically. All forms can attack foliage, floral parts, and the roots of wheat and barley. Some forms are very virulent while others are relatively non-virulent. In general, there is a correlation between the virulence on wheat and barley.

7. The differences in the pathogenicity of different forms are so great that the conflicting results obtained by previous investigators can easily be explained.

8. Many physiologic forms may occur in the same region. On the other hand, the same physiologic form sometimes may occur in widely separated regions.

9. Asexual mutation occurs frequently in some forms of *H. sativum*. Mutants arise abundantly from some forms in artificial culture as wedge- or fan-shaped sectors. These mutants bred true when propagated from either spores or mycelium.

10. Mutations occur as frequently in monosporous cultures as in those produced from many spores or from mycelium.

11. Some forms mutate readily and others do not. Mutation was observed in forms from England, Australia, Africa, Argentina, Serbia, Canada, and many localities in the United States.

12. Reversions apparently occur and when they do they are always in the form of sectors.

13. The mutants differ from their parents not only in morphological characters, but also in pathogenicity. Some are more virulent than the parents while others are less so.

14. Many physiologic forms of *H. sativum* produce conidia abundantly on plant tissues and artificial media; others seldom fructify. All attempts to induce formation of perithecia were unsuccessful.

15. Light is not necessary for the formation of spores. The production of conidia of *H. sativum*, Form 1, was affected greatly by temperature. Different physiologic forms react differently to temperature. The optimum temperature for sporulation for Form 1 was between 16° and 25° C. The maximum was about 29° and the minimum about 10°.

16. The conidia of *H. sativum* remain viable for a long time under proper conditions. Those of several forms remained viable for 3 years, while a successful transfer was made from an old culture after it had been dormant and dry for almost 5 years.

17. The longevity of the spores depends somewhat on the physiological form concerned, altho environmental conditions have a greater effect.

18. Spores of Form 1 can withstand exposure to both low and high temperatures for considerable periods of time if the relative humidity is low. High temperatures and high relative humidity are deleterious. The spores can withstand long periods of alternate freezing and thawing. The conidia of Form 1 overwintered outside at University Farm, St. Paul. The percentage of spores which survive the winter differs in different seasons.

19. Aeration is important in determining the length of time conidia can remain viable.

20. Spore germination is influenced profoundly by slight changes in environment.

21. The addition of small bits of leaf tissue to distilled water stimulates spore germination and growth of germ tubes to a remarkable extent. Spores kept in a liter of water for a year failed to germinate, but when suspended in a small quantity of this water to which leaf tissue was added, 80 per cent germinated within 24 hours. The stimulatory agent is present in other plant tissues than those of susceptible hosts.

22. The virulence of Form 1 was not attenuated as a result of growing six years on artificial media.

23. The development of the *Helminthosporium* disease on cereals depends on several factors: The physiologic forms present; the amount of inoculum produced; the effect of environmental factors on the development of the pathogene; varietal susceptibility; and factors predisposing the host.

24. Heavy dews and rains together with high temperatures are conducive to foliage and spike infection.

25. The disease was most severe on peat soil with deep drainage. In 1924 practically all plants of Monad and Pentad wheat were killed by root-rots and basal stem-rots on four different water levels (2 to 5 feet), but not on the 1-foot water level.

26. Peat soils are likely to predispose plants to attack, because the plants are weaker than those growing on better soils.

27. Plants seem to be most susceptible to the disease from the milk stage on.

28. While temperature and moisture undoubtedly affect the development of the disease, it is evident that the virulence of the particular form concerned is often more important than certain environmental conditions.

29. Many dust and liquid fungicides were used in an attempt to find something that would kill the mycelium of *H. sativum* in the seeds of wheat and barley. None were entirely effective.

30. Treating the seed with fungicides did not affect the rate of growth of seedlings, nor did it control root-rot and basal stem-rot or increase the yields when the seed was sown in soil in which the organism was present. All varieties of *Triticum vulgare, T. compactum, T. durum, T. dicoccum, T. turgidum, T. spelta,* and *T. polonicum* became infected with root-rot and basal stem-rot. There were great differences in susceptibility, however. The durums, in general, were the most susceptible. There were sharp differences in the susceptibility of different varieties also among the common wheats.

31. There is a high correlation between the behavior of varieties to *H. sativum* when grown at different places in the same year and at the same places in different years. This indicates clearly that resistance of wheat is due to genetic factors the expression of which can, however, be influenced considerably by the environment and by the specialization of the fungus. Very unfavorable growing conditions may predispose even the most resistant varieties of wheat and barley, so that they may be severely attacked by virulent forms.

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PLATE I

Fig. 1. Cultures of *H. sativum* and *Alternaria spp*. Developed from Barley Seed on Potato Dextrose Agar

Fig. 2. Pure Cultures of *H. sativum* Developed from Basal Portions of Diseased Stem of Marquis Wheat

PLATE II

Fig. 1. Heads of Barley Inoculated in the Boot Stage with Three Physiologic Forms of *H. sativum*

A with Form 1	C with Form 8
B with Form 5	D control

Fig. 2. Heads of Wheat Inoculated in the Boot Stage with Three Forms of *H. sativum*

A Control	C Form 9
B Form 1	D Form 25

PLATE III

Ten-Day-Old Cultures of 18 Forms of *H. Sativum* Grown Under Identical Conditions on Oatmeal-Rice-Cornmeal Agar. Compare with Plate IV

PLATE IV

Ten-Day-Old Cultures of 18 Forms of *H. Sativum* Grown Under Identical Conditions on Potato Dextrose Agar. Compare with Plate III

PLATE V

Fig. 1. Seedling of Marquis Wheat Grown in Soil Inoculated at Time of Planting with *H. sativum*, Showing the Comparative Virulence of Different Forms of the Pathogene

A Control	E Form 19
B Form 26	F Form 22
C Form 21	G Form 8
D Form 3	H Form 5

Fig. 2. Seedlings of Mindum Wheat Grown in Soil Inoculated at Time of Planting with Forms of *H. sativum* Obtained from Widely Different Localities

A Control	E From Canada
B From Argentina	F From Texas
C From Serbia	G From England
D From Australia	H From Minnesota

PLATE VI

Seedlings Grown in Soil Inoculated with Approximately Equal Amounts of Inoculum of Different Fungi, Showing the Comparative Virulence of Each

A Control	E *H. sativum*, Form 8
B *Gibberella saubinettii*	F *H. sativum*, Form 1
C *Fusarium sp.*	G *H. sativum*, Form 21
D *Fusarium moniliforme*	

PLATE VII

Fig. 1. *H. sativum,* Form 1, on Oatmeal-Rice-Cornmeal Agar, Showing Mutants

Fig. 2. *H. sativum,* Form 1, on Oatmeal-Rice-Cornmeal Agar, Slant Placed so that the Depth of Agar at the Bottom Was About Three Times That at the Top. Mutants Only on the Shallow Half of the Medium

PLATE VIII

Fig. 1. *H. sativum,* Form 31, Giving Rise to Similar Mutants

Fig. 2. *H. sativum,* Form 3, Producing Two Different Mutants, No. 51 (right) and No. 52 (left)

PLATE IX

Cultures Showing Homozygosity of *H. sativum,* Form 1, Obtained by Single-Spore Inoculations from a Monosporous Culture

Upper Row on Oatmeal-Rice-Cornmeal Agar; Lower Row, on Green Bean Agar

A Mass Transfer of Inoculum as Control
B-E Single-Spore Inoculations

PLATE X

Fig. 1. Seedlings of Marquis Wheat Grown in Soil Inoculated with *H. sativum,* Form 22 (back row), and Its Mutant, No. 40 (front row), Showing the Difference in Virulence of Parent and Mutant

Fig. 2. Seedlings of Trebi Barley Grown in Soil Inoculated with *H. sativum,* Form 22 (back row), and Its Mutant, No. 40 (front row), Showing Difference in Virulence of Parent and Mutant

PLATE XI

Mature Plants of Pentad Wheat Grown on Peat Soil at Two Different Drainage Levels, Showing Effect of Soil Moisture on the Development of Basal Stem-Rot

A One-Foot Drainage Level
B Three-Foot Drainage Level

PLATE XII

Colonies of *H. sativum* and *Alternaria spp.* Developing from Seed of Mariout Barley, Sown on Potato Dextrose Agar, After Treatment with Copper Carbonate (Fig. 1) and Silver Nitrate (Fig. 2).

H. sativum and *Alternaria spp.* Developed from all Seed Thus Treated
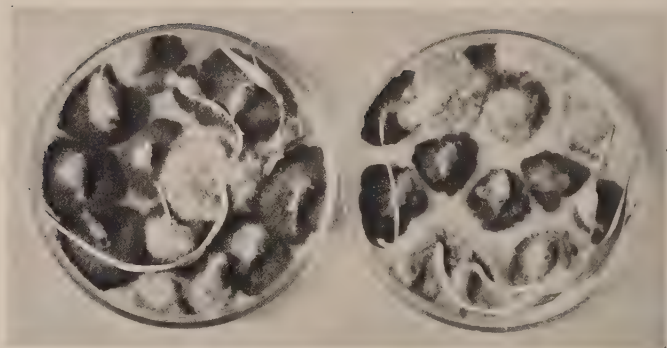
PLATE I

Figure 1

Figure 2

PLATE II

Figure 1
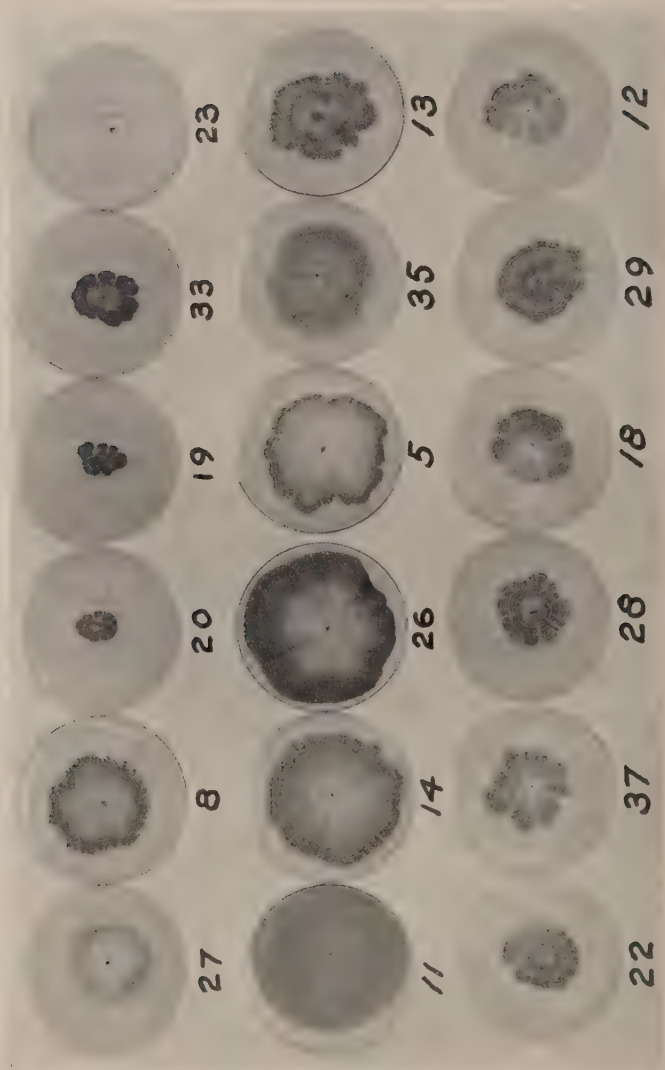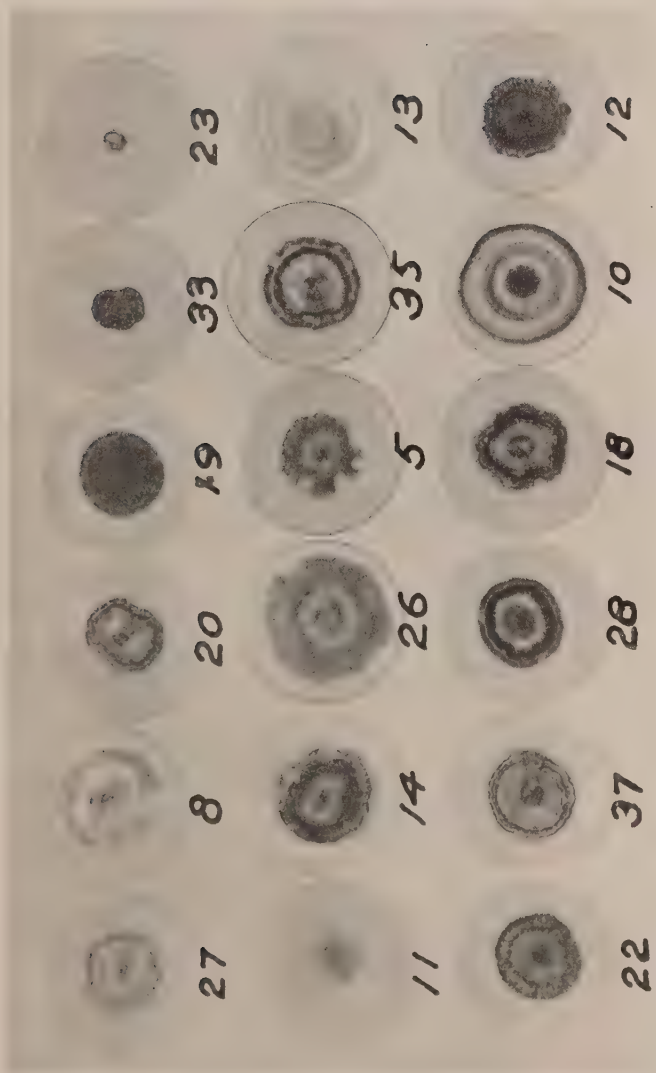

Figure 2

PLATE III

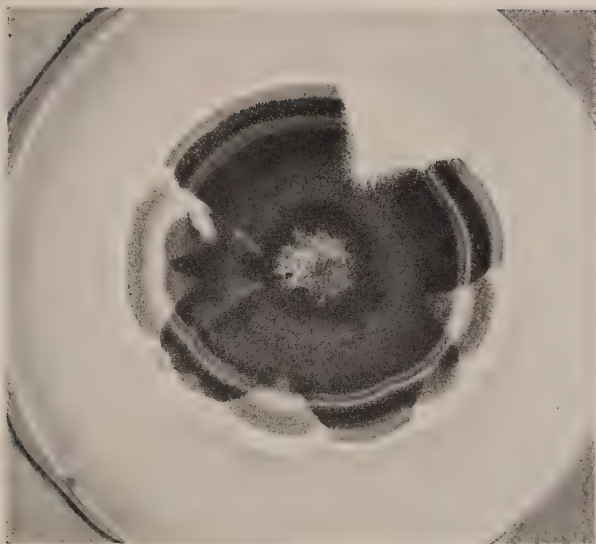PLATE IV

PLATE V

Figure 1

Figure 2

PLATE VI

PLATE VII

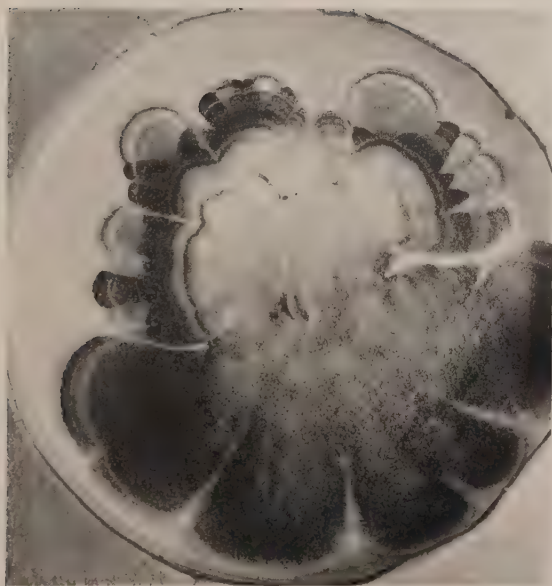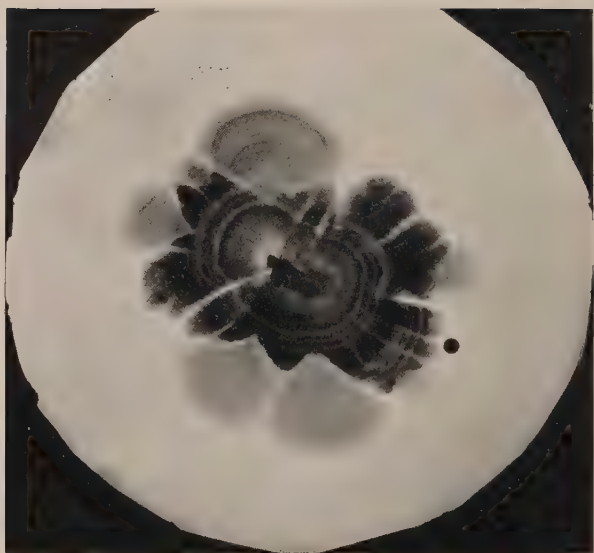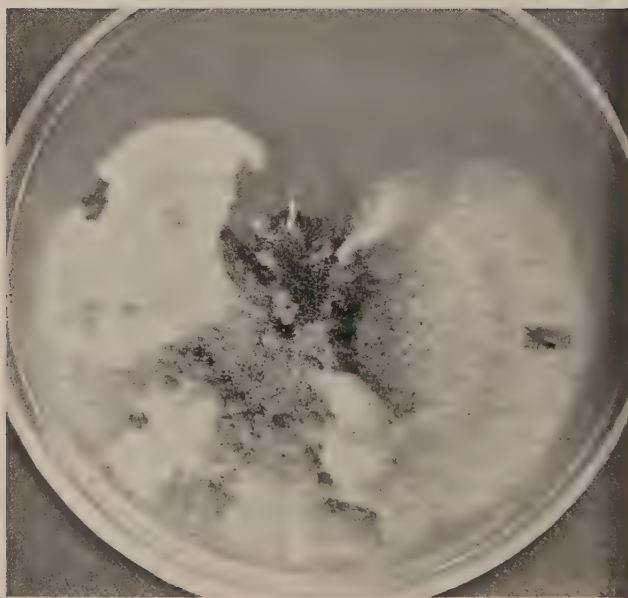
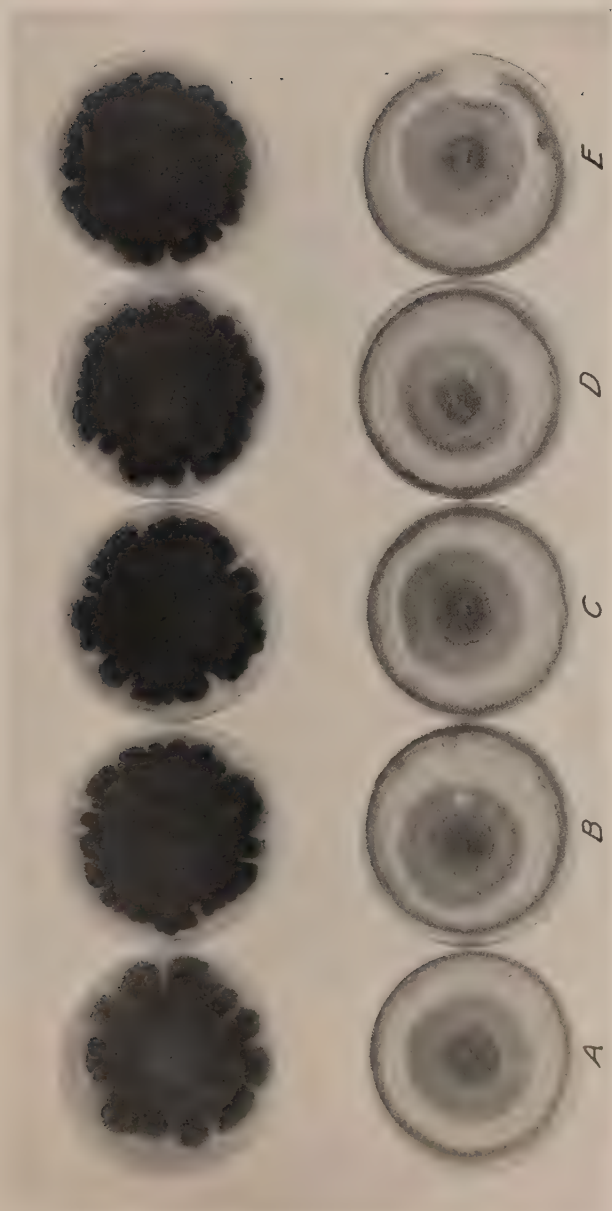
Figure 1

Figure 2

PLATE VIII

Figure 1

Figure 2

PLATE IX

PLATE X

Figure 1

Figure 2

PLATE XI

PLATE XII